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ÉTUDES SUR LES HYBRIDES DE CISTES

VI. INTROGRESSION DANS LA SECTION *LADANIUM*[1]

PAR PIERRE DANSEREAU[2]

Sommaire

Certaines méthodes appliquées à la taxonomie permettent d'apprécier le mode de la genèse des divers groupes. Des études précédentes ont défini le mécanisme de l'introgression: persistance après hybridation de certains caractères d'une espèce dans une autre. Ces notions appliquées à la section *Ladanium* du genre *Cistus* indiquent que le *C. ladaniferus* var. *petiolatus*, localisé en Afrique du Nord, a eu comme origine une contamination du *C. ladaniferus* par le *C. laurifolius*.

Identification des mécanismes de l'évolution

Le processus de la différenciation, qui a pour résultat l'élimination progressive des virtualités et la spécialisation de plus en plus étroite des formes vivantes, s'accomplit par des mécanismes divers agissant séparément ou ensemble et éprouvant de la part du milieu plus ou moins de résistance. La diversité des modalités mises en cause et de leur conjonction engendre des séries de variations non équivalentes sur lesquelles sont basées les entités taxonomiques.

La matière végétale a apparemment produit des formes nouvelles par quatre mécanismes principaux: mutation, polyploïdie, hybridation, sélection.* Les données que nous possédons actuellement sur la fréquence des mutations dans la nature sont sans doute insuffisantes pour permettre des généralisations, surtout en l'absence de la connaissance des causes naturelles susceptibles de les provoquer. Sugiura (14) a cependant trouvé que, sur 80 familles, représentées par 250 espèces, celles dont le nombre respectif est de n et $n + 1$ ou $n - 1$ manifestaient entre elles plus d'affinité que celles à n et un multiple de n. Les mutants aneuploïdes s'écarteraient donc moins du phylum primitif que les polyploïdes.

En fait, les mutations simples (sans polyploïdie ou aneuploïdie) sont accompagnées, la plupart du temps, de variations morphologiques peu importantes, portant sur des caractères qui engendrent des formes et des variétés seulement.

Tandis que, dans certains groupes le doublement du nombre chromosomique entraîne la ségrégation générique, spécifique, ou variétale, tandis que dans

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* *C'est là une classification surtout pratique puisque la mutation peut être génique ou chromosomique, (et peut donc inclure la polyploïdie, et que la sélection est une conséquence ordinaire de l'hybridation.*

d'autres groupes elle ne s'accompagne apparemment d'aucune variation morphologique (10).

L'hybridation se produit à tous les degrés de l'échelle taxonomique. On lui a donné le nom de métissage quand elle se manifeste à l'intérieur d'une espèce. Cette distinction ne vaut évidemment, à un point de vue strictement objectif, que ce que vaut l'espèce linnéenne elle-même. On constate que certaines variétés d'une même espèce ne se croisent pas; tandis que les variétés d'une autre espèce sont rendues à ce point instables par la fréquence de leurs croisements qu'on peut douter de leur existence et considérer l'espèce globale comme un tout délimité quant à l'extérieur, mais où la différenciation est trop peu avancée à l'intérieur pour l'avoir compartimentée (*Cistus salviifolius*) (8).

Il faut donc, pour qu'une entité taxonomique soit légitime, qu'elle ait une base naturelle reconnaissable aux barrières qui l'isolent morphologiquement et génétiquement des entités affines. Un croisement est théoriquement suivi d'un grand nombre de recombinaisons, associant les divers caractères des deux groupes originaux en des assemblages nouveaux. Il a été prouvé, dans la pratique, qu'un très petit nombre de ces possibilités se réalise, pour des raisons génétiques. Anderson a même formulé une loi de la recombinaison (2).

Il n'est pas moins clair que le milieu impose à son tour des limitations très étroites. La sélection opère tout de suite, et il ne semble pas douteux que, parmi les populations naturelles, le retour aux formes parentales originelles soit la règle. Ce retour est, dans certains cas, très rapide (1). Certains croisements entre espèces sont génétiquement aussi faciles et aussi fertiles que les fécondations légitimes (surtout dans le cas de plantes autostériles (7)). Dans le cas des *Cistus monspeliensis* et *salviifolius*, par exemple, le croisement est très fréquent. Certes, l'hybride auquel ces deux espèces donnent naissance (× *C. florentinus*) n'est pas rare dans la nature, sauf en Afrique du Nord, mais il n'atteint pas une importance numérique notable, sauf dans des conditions spéciales sur lesquelles nous aurons l'occasion de revenir*. Les recombinaisons sont nettement différentes selon les lieux (8). La sélection agit donc localement sur les populations végétales et élimine certains caractères en présence, soit qu'elle agisse sur une espèce pure dont elle utilise ce qui lui reste d'hétérozygotie, soit qu'elle choisisse dans le stock plus riche d'un hybride, toujours avec la tendance vers l'équilibre homozygote et la stabilité cyto-nucléaire. Ce travail peut être contrarié par la formation de nouveaux individus hybrides ayant la même formule et tendant à ramener toujours l'évolution que dirige la sélection à son point de départ, ou favorisé par la disparition accidentelle de certaine catégorie d'individus, par exemple de l'un ou des deux parents, s'il s'agit d'un hybride, ou de l'espèce-type s'il s'agit d'une espèce pure. C'est vraisemblablement le cas des *Pteridium latiusculum* et *aquilinum*, du *Pinus laricio* et de ses variétés où la barrière géographique a aidé le travail de la sélection et de la conservation locale des mutations (4).

* *Voir: Études sur les hybrides de Cistes. V. Comportement du* × *C. florentinus Lam. (en cours de préparation).*

Il n'est pas exagéré de prétendre que nous connaissons les voies de l'évolution et qu'il nous est possible de déceler le mécanisme ou les mécanismes formateurs d'entités nouvelles. Il est intéressant de noter, ici comme ailleurs, que le travail accompli est sans relation nécessaire avec l'importance du résultat et il semble qu'il ne faut pas plus de travail pour produire une forme qu'une espèce. Quant à l'action de la sélection naturelle, elle est à la fois plus aléatoire et plus difficile à observer, puisque, dans presque tous les cas, le facteur "temps" dépasse de beaucoup l'échelle de nos observations.

La sélection agissant au sein de groupes complexes comme les *Rosa*, *Rubus*, *Mentha*, *Euphrasia*, n'est guère décelable. Par contre, elle devient sensible dans les groupes moins riches en espèces, moins polymorphes ou, en tous cas, dans des groupes où sont en jeu un certain nombre de caractères qualitatifs, et en présence de zones de discontinuité strictement observables.

Les auteurs ont émis des opinions diverses sur l'importance de l'hybridation dans l'évolution. Lotsy, par exemple, lui assigne le rôle principal (11). Mais, comme le faisait remarquer Anderson (1), cette importance ne sera mesurable que lorsque nous aurons acquis une connaissance concrète des effets de l'hybridation dans la nature. Il importe donc surtout, pour l'instant, d'accumuler des documents sur cette question, en vérifiant la constance du mécanisme dans divers groupes taxonomiques. La statistique des populations naturelles et l'étude cytologique des hybrides sont appelés à nous fournir les plus précieux indices sur les relations de l'hybridation et de la sélection.

La signification de l'introgression

Un aspect particulier de la sélection naturelle est le mécanisme de l'introgression, défini explicitement par Anderson dans une étude sur les hybrides de *Tradescantia* (1). Ce phénomène a été constaté depuis chez d'autres genres: *Aster* (15), *Iris* (12), et *Cistus* (5). L'introgression est le passage d'un ou de plusieurs caractères d'une espèce dans une autre à la suite d'hybridations. C'est un mécanisme qui favorise la différenciation d'un certain nombre d'individus d'une espèce dans un sens qui les rapproche d'une autre espèce existante. Il induit donc la convergence et se présente dans la phylogénie comme une anastomose.

Certes, les cas d'évolution parallèle ne sont pas rares et chaque fois qu'on trouve un caractère d'un groupe dans un autre groupe, il est imprudent de conclure à l'hybridation. Ne voit-on pas des *Populus* européens et américains produire des formes analogues à partir eux-mêmes de types peu divergents? De même les formes épineuses et inermes chez les *Rosa* de l'Amérique du Nord résultent aussi vraisemblablement des gènes latents dans le genre entier ou de mutations habituelles (voir aussi le feuillage panaché chez l'*Acer negundo*) que de l'hybridation. Pour d'autres cas, le rôle de l'hybridation est évident, comme pour l'évolution parallèle des *Circaea* européens et américains.

Anderson a proposé un système qui permet de mesurer la gravité de l'influence exercée par l'hybridation, en convertissant les données qualitatives

sous une forme quantitative (1). Il a fourni lui-même une première application de son principe en analysant des populations naturelles du *Tradescantia canaliculata*, du *T. virginiana* et de leurs hybrides.

L'expérience d'Anderson a été répétée par Riley sur des *Iris* (12) et des *Tradescantia* (13) de la Louisiane, par Wetmore et Delisle sur des *Aster* (15), par Goodwin sur des *Solidago* (10), et par l'auteur sur des *Cistus* (5).

Les résultats obtenus jusqu'ici permettent de donner à une espèce une expression mathématique et graphique, au moins en ce qui concerne sa position relativement à une autre espèce. Ils permettent, en outre, d'apprécier le degré d'éloignement des individus hybrides par rapport à chacun des parents, et de constater lequel des deux est favorisé dans la recombinaison. Dans la plupart des cas, l'un est à ce point favorisé que ce qui reste de l'autre après la recombinaison est trop insignifiant pour mériter un rang taxonomique (3).

Aucun des auteurs précités n'a voulu accorder un status précis aux spécimens non-typiques ou non franchement intermédiaires. En ce qui concerne le *Cistus monspeliensis* l'auteur, n'a pas voulu non plus accorder de valeur taxonomique à l'introgression de quelques petits caractères du *C. salviifolius* (5).

Il semble probable qu'il faut, pour qu'un caractère introgressif se fixe, la collaboration d'autres causes, la disparition accidentelle de populations pures susceptibles de *noyer* ce caractère ou la répétition, pendant une période très longue, du même phénomène avec élimination graduelle des autres types. Je crois que c'est ce dernier cas qui se présente dans la section *Ladanium* du genre *Cistus*.

Dynamisme de la section *Ladanium* du genre *Cistus*

Cette section se compose de deux espèces, les *C. laurifolius* et *ladaniferus* (6).

Le *C. laurifolius* L. a des feuilles longuement pétiolées, le plus souvent cordées, très larges (18 à 30 mm.), à marge le plus souvent ondulée. Son inflorescence porte cinq à huit fleurs en ombelle, à pétales blancs de 20 à 30 mm. de longueur; la pubescence du calice se compose de poils simples assez longs; les loges de l'ovaire sont au nombre absolument invariable de cinq. Il se trouve en des stations isolées en Asie Mineure, en Italie, en France méridionale, et assez abondamment en Espagne et au Portugal (Fig. 3).

Le *C. laurifolius* L. var. *atlanticus* Pit. (Atlas et Corse) (Fig. 3) diffère du type par des caractères exclusivement quantitatifs, i.e., feuilles et sépales plus petits, etc.

Le *C. ladaniferus* L. typique (var. *albiflorus* Dun. emend P. Dans.) a une inflorescence solitaire, faible et plus ou moins retombante, couverte de larges bractées; les sépales ne portent pas de poils simples, mais des poils étoilés d'une forme très spéciale dite "en écusson"; les loges de l'ovaire sont au nombre de 10; les pétales sont très grands (30 à 35 mm.) et portent parfois une macule pourpre à la base. Les feuilles sont sessiles, linéaires ou lancéolées, larges de

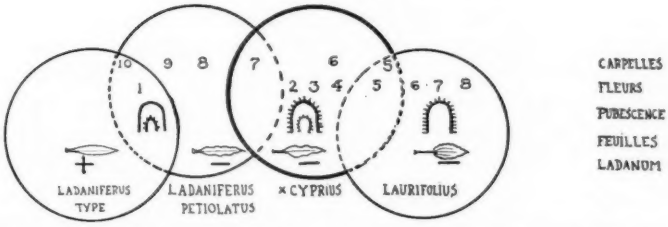

FIG. 1. *Répartition des caractères dans la section Ladanium du genre Cistus. Nombre de carpelles (de 5 à 10); nombre de fleurs (de 1 à 8); pubescence simple (à droite, chez le C. laurifolius), en "écusson" (à gauche, chez le C. ladaniferus) ou des deux types (× C. cyprius); feuilles sessiles (C. ladaniferus type) à longuement pétiolées (C. laurifolius); feuilles planes (C. ladaniferus type) à fortement ondulées (C. laurifolius); présence (+) ou absence (−) de ladanum.*

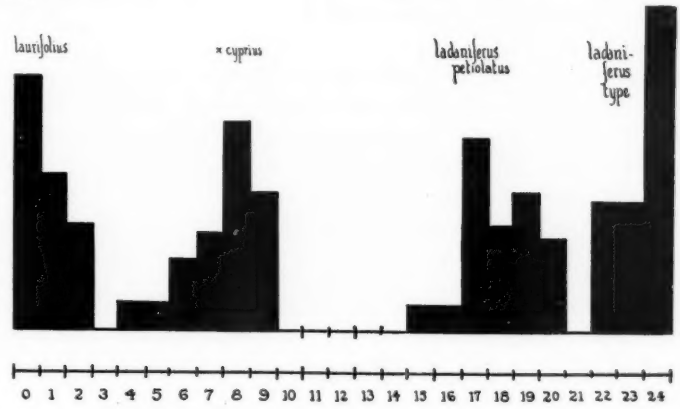

FIG. 2. *Profil statistique des quatre groupes constituant la section Ladanium. Des caractères qualitatifs ont reçu une valeur quantitative permettant d'établir les positions relatives des quatre groupes taxonomiques.*

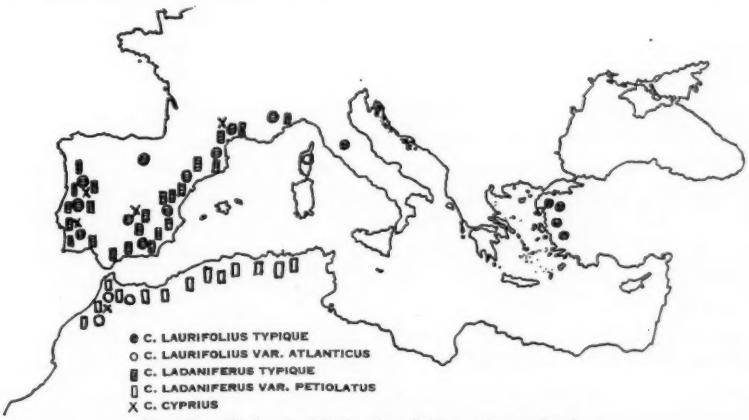

FIG. 3. *L'aire de distribution de la section Ladanium.*

10 à 21 mm. à marge plutôt plane, enduites d'une résine appelée "ladanum". Il est très répandu en Espagne et au Portugal et on le trouve localement en France méditerranéenne.

Les *C. ladaniferus* de l'Afrique du Nord appartiennent tous à la variété *petiolatus* Maire, qui diffère du type par les caractères suivants: loges à l'ovaire 7 à 10, feuilles courtement pétiolées, plutôt ondulées, sans ladanum.

Le × *C. cyprius* Lam. (*C. ladaniferus* × *C. laurifolius*), d'autre part, peut se reconnaître à ses 5 à 7 loges à l'ovaire, ses sépales portant les deux types de pubescence mentionnés plus haut, à ses fleurs dont le nombre varie de deux à cinq, à ses feuilles pétiolées assez longuement et de contour plus ou moins ovale, ne sécrétant pas de ladanum. On le rencontre fréquemment dans les jardins et on l'a souvent trouvé à l'état sauvage, en Europe et en Afrique, dans la zone habitée par les espèces parentes.

La Fig. 1 fait voir les associations de caractères telles qu'on les trouve réalisées dans la nature. Les combinaisons de facteurs sont si loin d'être libres que les quatre entités principales de la section *Ladanium* manifestent une parfaite discontinuité et se révèlent extrêmement faciles à caractériser. Les frontières indiquées pour chaque groupe sont absolues et basées sur l'observation d'un grand nombre de spécimens.

La Fig 2 est une transposition de la Fig. 1, basée sur la méthode statistique citée plus haut. Les pôles sont, d'une part le *C. laurifolius* et d'autre part le *C. ladaniferus* typique. Sept séries de caractères ont été considérées; une valeur relative a été donnée à chaque modalité de chaque série, élevée si elle faisait partie du complexe "*ladaniferus*", nulle si elle faisait partie du complexe "*laurifolius*" et plus ou moins élevée selon qu'elle se rapprochait ou s'éloignait du pôle "*ladaniferus*". Le tableau I énumère ces caractères et donne la valeur qui leur a été assignée dans la Fig. 2.

La valeur idéale du *C. laurifolius* sera donc de O, et celle du *C. ladaniferus* typique de 24, ceci étant donné que les caractères mentionnés sont absolus pour chaque espèce. Les caractères de feuilles, cependant (séries V et VI) sont un peu moins absolus, et dans l'ensemble des spécimens que j'ai pu examiner c'est faire amplement la part des choses que d'admettre que 40% ont la marge ondulée; et que d'admettre, d'autre part, que 29% des *C. ladaniferus* européens ont une marge ondulée et 20% une feuille de 5 à 10 mm.

Etant donné la valeur constante des séries I, II, III, IV, et VII pour les *C. laurifolius* et *ladaniferus* var. *albiflorus*, on peut admettre les figures ci-dessus comme profil de ces groupes taxonomiques, au moins en ce qui concerne les caractères qui les séparent, sans recourir à un examen statistique qui ne saurait donner un résultat sensiblement différent de celui que je propose. La valeur de 0 pour les deux séries (V et VI) n'a jamais été observée chez un *C. ladaniferus* typique et même une valeur de 1 pour chaque série chez un grand nombre de *C. ladaniferus* var. *albiflorus* ne changerait pas sensiblement la position de ce groupe sur le graphique, puisqu'on n'enregistrerait en aucun cas de valeur inférieure à 22. De même pour le *C. laurifolius* qui ne s'écarte

de la valeur 0 que pour la série V, ce qui rend impossible pour lui toute valeur supérieure à 2. Les deux masses, donc, qui occupent la droite et la gauche de la Fig. 2 constituent chacune un ensemble cohérent et constant et expriment la réalité de tous les individus des groupes en question. (Voir description (6).)

TABLEAU I

Numéro de la série	Caractères	Valeur relative
I	Nombre des carpelles	
	10	4
	9	3
	8	2
	7	1
	6	1
	5	0
II	Nombre de fleurs	
	1	4
	2–4	2
	+4	0
III	Poils des sépales	
	en écusson	4
	des deux types	2
	simples	0
IV	Longueur des pétioles	
	nulle	4
	–5 mm.	2
	5–10 mm.	1
	10–25 mm.	0
V	Marge des feuilles	
	plane	2
	ondulée	1
	crépue	0
VI	Largueur des feuilles	
	1–5 mm.	2
	5–10 mm.	1
	10–20 mm.	0
VII	Présence de ladanum	
	présent	4
	absent	0

Quant aux deux autres groupes déjà représentés dans la Fig. 1, ils ont été l'objet, pour tous les caractères mentionnés, d'un relevé statistique. La position qui leur est assignée ici résulte d'observations faites sur 18 spécimens du × *C. cyprius* (*C. ladaniferus* × *C. laurifolius*) et 54 spécimens du *C. ladaniferus* var. *petiolatus*, le tout étant rapporté à 100 sur le graphique.

La première conclusion qui se dégage de cette figure est la parfaite discontinuité qui existe entre les quatre entités. Quand on songe qu'il s'agit de sept séries de caractères, cette discontinuité est sans doute très remarquable

et en tout cas suffisante pour permettre d'individualiser les groupes, de leur conférer une identité.

La position du × *C. cyprius*—40% portant l'indice 8—est assez significative au point de vue génétique, puisqu'elle nous indique que, dans l'ensemble, les caractères du *C. laurifolius* sont dominants. On voit en effet sur la Fig. 1 que jamais le × *C. cyprius* n'a plus de sept loges à l'ovaire; qu'il n'a jamais moins de deux fleurs; que ses feuilles sont toujours pétiolées et toujours plus larges que de 5 mm. Le matériel examiné était nord-africain aussi bien qu'européen, et cependant les valeurs sont essentiellement les mêmes puisqu'aucun spécimen n'a révélé un indice entre 10 et 14. Le matériel africain a toutefois donné les plus hautes valeurs pour les séries I et II.

Quant au *C. ladaniferus* var. *petiolatus*, s'il n'a aucun point de contact avec le type, il est, d'autre part, totalement dégagé de la zone d'hybridation hors de laquelle aucune plante ne saurait être distinguée de l'un ou de l'autre parent. Si on ne connaissait la valeur dominante de certains facteurs du *C. laurifolius*, on pourrait théoriquement étendre cette zone jusqu'à l'indice 12; mais même alors, on n'atteindrait pas le niveau inférieur des *C. ladaniferus* de l'Afrique du Nord. La question ne se pose pas, d'ailleurs, si l'on peut considérer ce groupe comme hybride puisqu'il constitue de vastes populations en Afrique du Nord, en l'absence totale du type (*C. ladaniferus* var. *albiflorus*) et qu'il s'hybride à son tour, avec le *C. laurifolius* et donne naissance à des × *C. cyprius* ayant un indice de 8.

L'hypothèse donc qui se présente le plus naturellement est la suivante. En Afrique du Nord, le *C. ladaniferus* a été contaminé, à un moment de son histoire, par le *C. laurifolius*. Cette hybridation et les recombinaisons subséquentes ont engendré la formation d'un groupe nouveau, retenant quelques-uns des caractères du *C. laurifolius*. ou plus exactement des caractères intermédiaires entre les deux espèces. Ce groupe nous apparaît aujourd'hui complètement stabilisé, puisqu'il n'existe plus d'individus appartenant au *C. ladaniferus* typique, en Afrique du Nord. Il se rapproche du *C. laurifolius* sans que cet écart soit assez grave pour le faire sortir de l'orbite de l'espèce-mère (*C. ladaniferus*).

La section *Ladanium* est donc un complexe bipolaire où la différenciation s'est faite au moyen de l'introgression.

Ce phénomène est sans doute assez commun dans les genres où les espèces s'hybrident facilement. Il s'est produit chez les *Tradescantia*, les *Iris*, et les *Aster*, et chez les autres espèces de *Cistus*. Mais on n'a pas signalé jusqu'ici que ce mécanisme ait engendré une entité taxonomique nouvelle, du même ordre de grandeur et aussi indiscutable que celle-ci. Riley, par exemple, n'approfondit pas l'identité taxonomique des *Iris* ayant des indices intermédiaires entre les *I. fulva* et *I. hexagona* var. *giganticoerulea* (12). Il n'est surtout jusqu'ici question nulle part de variétés à la fois morphologiquement bien caractérisées et géographiquement isolées, s'étant stabilisées par la sélection naturelle.

Il deviendra sans doute intéressant d'appliquer la présente méthode à beaucoup de variétés et même d'espèces décrites et acceptées par les taxonomistes. L'auteur ne croit pas que l'origine hybride, même relativement récente, infirme la validité d'un groupe taxonomique. Il pense à des formes comme les *Populus alba* var. *denudata*, *Cornus canadensis* var. *intermedia* et à des entités analogues qui se rapprochent visiblement d'une espèce voisine, quoique on puisse les considérer comme fixées à l'heure actuelle. Il n'est pas douteux qu'une investigation systématique d'un grand nombre de ces cas apporterait une contribution susceptible de donner des bases plus objectives à la phylogénie.

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PURPLE DWARF, AN UNDESCRIBED POTATO DISEASE, IN ALBERTA[1]

By G. B. Sanford[2] and S. B. Clay[3]

Abstract

The characteristic symptoms of what seems a new disease of potato, observed in Alberta, have been outlined, certain observations and preliminary experiments reported, and the name "purple dwarf" suggested for it.

Purple dwarf is perpetuated through the tubers, and the symptoms on plants from diseased tubers either appear immediately the plant emerges from the ground or apparently fail to become recognizable during the remainder of the season.

Apical growth is checked, the entire plant becomes stunted, the newer leaves develop a purplish hue along their margins and curl upwards, the phloem of the entire plant is disorganized, and a well developed, brown, dendritic necrosis extends from proximal to distal ends of the tuber. First the roots, then the stolons, and later the base of the stem, become brown and soon decay. Usually the central pith in the tuber and upper stem remains normal.

Purple dwarf was transmitted to healthy plants by grafting, a result which suggests that the causal agent is a virus.

Introduction

A potato disease, apparently different from any yet described, has been observed in Alberta during the past decade. However, the data are not yet adequate to indicate whether this disease is increasing. Specimens have been found in many potato fields in the southern, central, and north-central sections of Alberta.

The disease seems to develop equally well in all districts. The number of affected plants rarely exceeds 1%; many fields appear to be free from the disease. On the other hand, there has been recorded a few special cases in which the proportion of diseased plants is as high as 3 to 5%. All potato varieties seem to be susceptible. Although the malady has not yet seriously reduced yield, its early recognition and control is of much importance.

Following the preliminary observations, a more extensive study of the malady was begun in 1938, and sufficient data is now available to justify a preliminary description of this disease, for which the name "purple dwarf" is proposed.

Description

External Plant Symptoms

Symptoms of purple dwarf on the potato are, in some respects, similar to those listed by Barrus and Chupp (2), Muncie (3), and by Walker and Larson (5) for yellow dwarf, and also to those listed by Orton and Hill (4) for the blue stem disease of potatoes.

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Although the external plant symptoms vary considerably, they seem to be within a rather well defined pattern. Usually purple dwarf plants can be easily recognized as they emerge from affected sets (seed pieces) planted under field or greenhouse conditions. The entire plant is more or less stunted, rigid, rather brittle, and often of a darker green hue than healthy plants (Plate I, *A*). Frequently the sprouts from severely affected sets do not reach the surface, for they may remain abortive and eventually die. General stunting and distortion of the plant and early development of purple colour, especially on the margins of the apical leaves, are constant symptoms under field conditions. Plate I, *B*, illustrates the appearance of a plant in which the symptoms developed more slowly than usual.

The young leaves are dwarfed, typically wrinkled, and cupped. A definite, but usually not extensive, rosette growth of foliage is common at the upper nodes, and this may or may not be accompanied later by tuberous swellings of axillary shoots or stunted leaves. The petioles of the primary leaves usually are dwarfed and stiff, often slightly swollen, and curved upwards. The main stems do not assume a zig-zag direction or characteristically swell at the nodes. Some of the lower leaves of the plant may eventually become slightly purplish or yellowish along the margins and roll inward, but when this stage is reached, chlorotic areas between the veins of the leaves soon appear and death of the foliage and of the characteristic dark green stalks gradually proceeds. Sudden wilting of the plant does not occur, except when severe stem rot, caused by other agencies, develops.

All the underground parts of a plant showing early symptoms of purple dwarf are at first apparently normal, but soon the older roots, then the stolons, and finally the epidermis of the lower stem, turn brown and decay. This general browning begins at the extreme base of the stem and proceeds outward through the roots and stolons and upward through the stem (Plate II, *A*). The outer portion of these brown roots and stolons may be slipped off with ease. Plants from infected sets that show definite purple dwarf symptoms rarely yield tubers more than one inch in diameter; usually only a very few small ones, or none, develop. These tubers, which always show a severe necrosis, are not characteristically cracked or off-type, as is often the case in yellow dwarf. The stolons and tubers do not produce secondary growth. The parent set usually remains sound throughout the season.

Internal Plant Symptoms

Pith

Under field conditions, the pith of the stem of purple dwarf plants is at first normal to dark green in colour, and sound. It remains in this state until the disease is well advanced. However, after a few days a general disorganization may begin at the base of the stem, as a result of invasion by bacteria and fungi. This decay gradually extends upward in the pith for a distance of from two to three inches (Plate III, *A*). An occasional small local rusty area in the pith of the stem may occur in certain badly diseased

plants, but in most instances it is absent. The pith in the stolons and tubers is apparently normal. Therefore, in contrast to yellow dwarf (5), pith necrosis alone is not a reliable diagnostic symptom of purple dwarf.

Xylem

Examination under a microscope of a cross-section of the stem of a plant having typical purple dwarf symptoms indicates that the vessels are free from obstruction and apparently normal (Plate III, *A* and *C*). However, in a few instances where affected phloem strands were adjacent to these vessels, a slight discoloration of the inside wall of certain vessels occurred. This generally normal condition of the xylem is maintained throughout the entire plant. On the other hand, if a longitudinal section of the stem (Plate III, *E*) is viewed with the unaided eye, the general vascular area is seen to be more prominent and more discoloured than in uninfected stems, increasingly so toward the base. The discoloration mentioned occurs in the phloem strands on both sides of the xylem (Plate III, *C*, *G*, and *H*).

Phloem

The phloem throughout stems, stolons, and roots of purple dwarf plants is discoloured and often plugged with a substance that stains deeply with Sudan III (Plate III). Occasionally some of the sieve tubes are distorted and large. The walls of the phloem parenchyma also become thickened and stain with Sudan III. In young tubers the secondary phloem groups adjoining the storage parenchyma are usually more prominent than the outer circle of phloem groups adjacent to and in the cortical tissue. The necrotic phloem strands throughout the broad band of storage parenchyma in tubers are also more or less plugged and brown. Thus, in cross or longitudinal section, it is these necrotic phloem strands that form the typical netted pattern in affected tubers. (Plate III, *B* and *F*). As a rule the starch grains in the immediate vicinity of these necrotic strands, in tubers, are small or absent.

Another easily recognized symptom of purple dwarf, is a marked browning of the exterior of the vascular cylinder in the lower stem, stolons, and roots. This discoloration, which is revealed when the cortical and epidermal tissues are peeled back, may often resemble the browning associated with *Fusarium* or *Verticillium* wilt. In cases where the disease originates from a diseased set, the discoloration begins at the base of the stem and proceeds upward in the stem and outward in the roots and stolons. In a few days this change is accompanied by the accumulation of excess moisture. At this stage, bacteria are present, but fungi are rarely isolated. The brown exterior of the central stele of affected plants is illustrated in Plate II, *B*. Further, the browning just described is most pronounced at the nodes where the roots and stolons leave the stem (Plate II, *B*). This might be expected in view of the greater development of the phloem at this point (1).

Cortex

The cortex of the lower stem, stolons, and roots retains its natural appearance for a short time after browning of the phloem occurs, then becomes discoloured and finally disintegrates. In the roots, complete disintegration of the endodermis and cortex may take place relatively early, but in the stolons, and occasionally in the lower stem, this is more gradual.

Purple Dwarf Transmitted Through Tubers

The results from experiments and extensive field observations leave no doubt in the minds of the authors that purple dwarf is transmitted through affected tubers. To date all definite purple dwarf plants appearing in commercial potato fields have originated from tubers that carried the purple dwarf disease. A few of the typical examples observed will be cited to illustrate this connection.

In one plot of about one-third acre, planted for foundation material with selected stock by the tuber-unit method, there were three units in which purple dwarf developed. All four sets in one unit produced typical purple dwarf plants, each with different degrees of severity (Plate IV, *A*). This is not uncommon. In the second unit three plants were healthy and one had purple dwarf. The third unit produced three purple dwarf plants and a healthy one. This apparently healthy plant, which was transplanted to the laboratory field plots, did not develop purple dwarf symptoms. None of the sets of the three affected units had internal net necrosis.

In a nearby commercial field (approximately two acres) of Netted Gem potatoes, 11 typical purple dwarf plants were observed during early July. The sets of these plants did not have internal net necrosis. Also, no other plants in either of the two fields mentioned developed purple dwarf symptoms during the season.

In another foundation plot of about one-half acre in size, and located 30 miles distant, there were eight tuber units in which no plants emerged, and one in which four plants emerged and all had purple dwarf symptoms, but no tubers were produced. The plants in all other unaffected units in the plot were apparently normal throughout the season. Internal net necrosis was apparently absent in the sets that produced the purple dwarf unit just mentioned, and also absent in the sets of all but two units of those that failed to produce plants.

Many other cases could be mentioned to illustrate that the purple dwarf plants, which appear early in potato fields, originate from random tubers that carry this disease. Also, the available evidence indicates that in many instances the sets that produce purple dwarf plants are apparently free from internal net necrosis. On the other hand, it might be expected that some net necrosis would be found in the parent tubers, because whenever tubers are formed on plants with definite purple dwarf symptoms they apparently always develop severe internal necrosis.

Disease Develops Early

Present evidence indicates that either the disease shows up early in a pronounced and definite form, as soon as the plants from affected tubers emerge from the ground, or not at any time later during the season. In view of this situation one suspects that the initial symptoms of purple dwarf, from possible field infection later in the season, must be slight or masked. Obviously the identification of these initial symptoms is an important factor affecting control measures.

Experimental Results

Transmission of Purple Dwarf

During the present season, attempts were made to transmit purple dwarf to healthy potato, tobacco, and tomato plants. One method was to place in the stem of a healthy plant a short length of a petiole, with leaflet attached, taken from an affected plant. Another method was to transfer a thin disc from the stem of an affected plant to an incision made in the stem of a healthy plant, or to one made near the semi-dormant eye of a healthy potato set. Also, a thin slice was aseptically removed from the eye of potato sets that had produced purple dwarf plants, and buried near the eye of a healthy set. The grafts were waxed to prevent loss of moisture. These various experiments, and results obtained from them to date are listed in Table I.

The data in Table I indicate that in potatoes purple dwarf can be transferred by inserting a short length of a petiole from an affected plant into the stem of a healthy plant. One of these purple dwarf plants produced by grafting is illustrated in Plate IV, *B*. The small tubers had severe internal

TABLE I

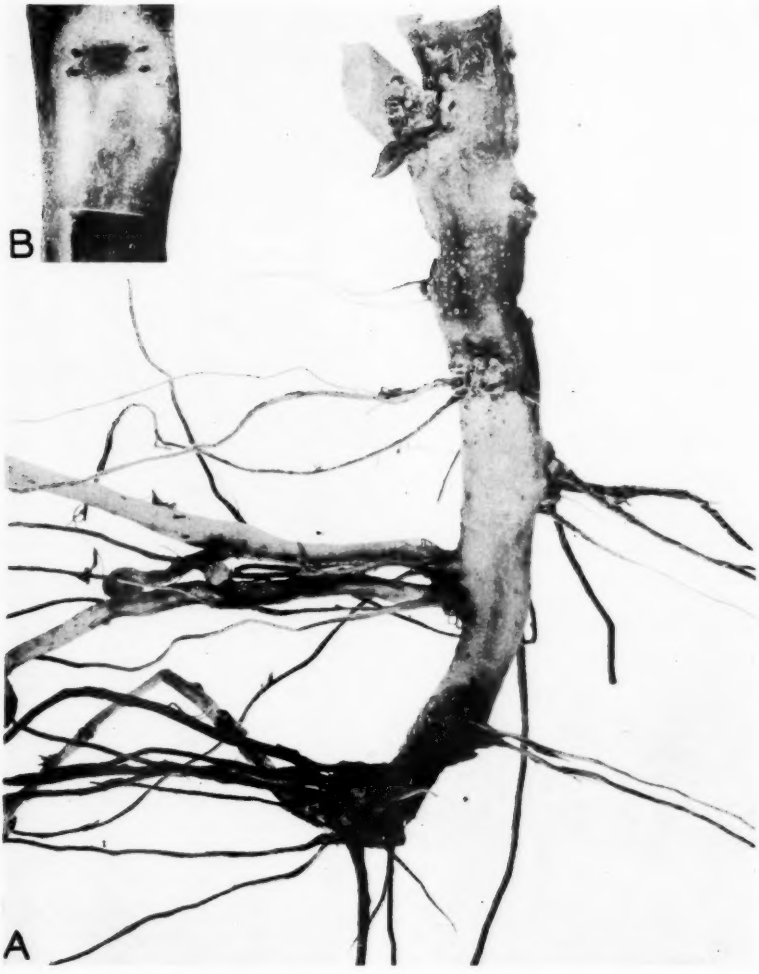
Results of inserting purple dwarf tissue into healthy potato, tomato, and tobacco plants

Expt.	Source of purple dwarf tissue		Grafts				Number of plants, %		Number of checks
	Variety	Origin	Variety	Place	Plants	Date	Positive[1]	Negative	
1	Warba	Petiole	Warba	Stem	13	6/7	7	6	13
2	Warba	Petiole	Ohio	Stem	6	6/7	0	6	6
3	Warba	Base[2]	Ohio	Set[3]	15	6/7	0	15	10
4	Gem	Base	Warba	Stem	9	12/7	2	7	9
5	Gem	Base	Warba	Set	22	17/7	0	22	10
6	Gem	Old set	Warba	Set	9	17/7	0	9	10
7	Warba[4]	Petiole	Tomato	Stem	*10*	7/8	*0*	*5*	5
8	Warba[4]	Petiole	Tobacco	Stem	4	7/8	3[5]	1?	4

[1] *Typical purple dwarf symptoms.*
[2] *Base of stem.*
[3] *Tissue inserted adjacent to eye.*
[4] *From Experiment No. 1.*
[5] *Plants stunted, leaves mottled, and area surrounding veins dark green.*

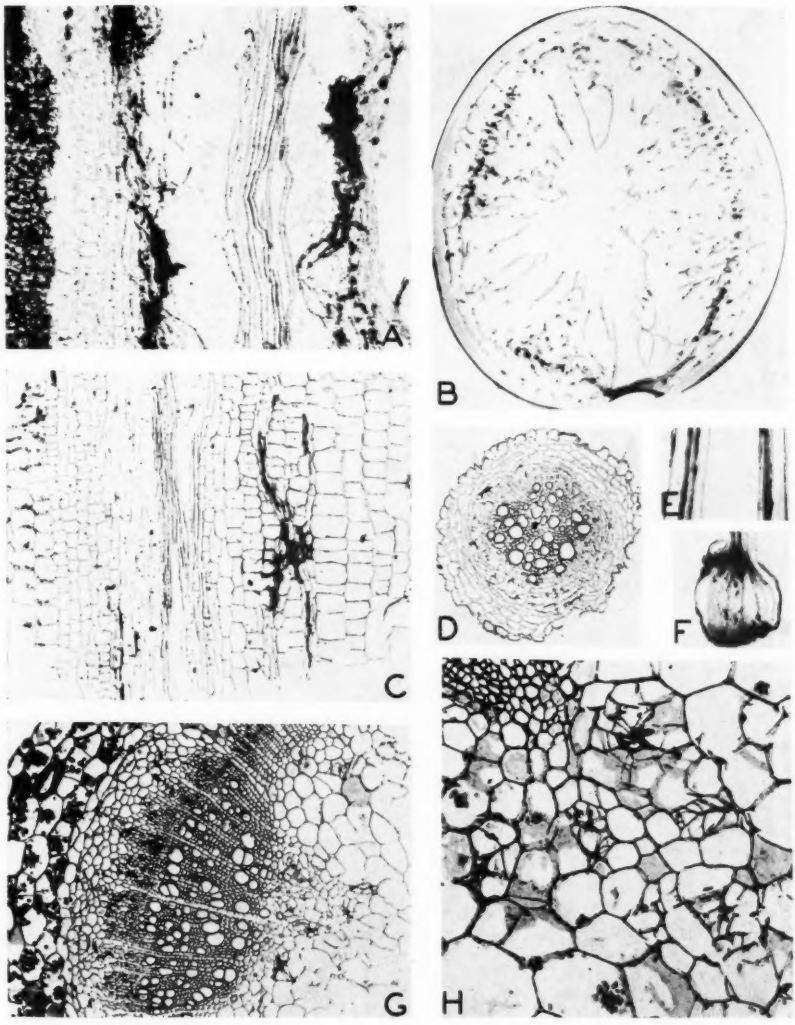
A. Typical purple dwarf symptoms soon after emergence. The lower set of roots are dead.

B. The symptoms developed more slowly in this plant.



A. Lower stem, roots, stolons, and small tubers of a purple dwarf plant. Some roots and stolons are more affected than others and the end of the stem is rotting.

B. Brown interior of roots and stolons at node, also the exposed brown central stele below.



A. Longitudinal section of tuber. Note, left to right, normal periderm and cortex, affected external phloem, normal xylem, and diseased internal phloem.

B. Cross-section of tuber in A. Note normal central pith and wide distribution of affected phloem groups.

C. Longitudinal section of stem at ground level showing diseased phloem elements and normal cortex and xylem. Note outer phloem groups are less prominent than the inner ones.

D. Cross-section of root of purple dwarf plant showing affected phloem and disorganization of adjacent cells.

E. and F. Longitudinal sections of stem and tuber of purple dwarf plant showing (E) brown vascular region in stem and (F) brown phloem strands throughout young tuber with massing at stem end.

G. Cross-section of stem in C. Note internal phloem groups are more prominent than external phloem groups adjoining cortex.

H. Affected groups of inner phloem in G, magnified.

A. All four plants of this unit have typical purple dwarf symptoms. Note differences in development and also the healthy unit in background.

B. One of the plants which developed typical purple dwarf symptoms as a result of grafting experiments. (See Table I and context.)

net necrosis. Although slightly more than 50% of the grafts were successful in Experiment 1, and only 22% in Experiment 4, it is now known that a much greater degree of success is possible.

It is important to note that the disease was apparently not transferred by inserting tissue from potato sets that had produced typical purple dwarf plants into the eyes of healthy sets. Negative results were also obtained with stem tissue. However, there is the possibility that purple dwarf will develop from the progeny of the plants when planted next season.

So far, negative results only were obtained from the grafts made on tomato plants. Further attempts will be made. However, from the grafts on tobacco *(Nicotiana tabacum)* were produced stunted plants with mosaic on the foliage. The effect was definite and uniform for each plant.

The results of the experiments listed in Table I indicate that much more can be learned, by continuing this type of work, about the nature and behavior of purple dwarf, especially whether the potato plant can carry the disease without developing recognizable symptoms in the field.

Etiology

The virus nature of purple dwarf is suggested because the disease originates from affected tubers, the tissue of which yields neither bacteria nor fungi when cultured on potato dextrose agar, and also by the fact that typical purple dwarf symptoms have been reproduced experimentally within approximately 30 days by grafting diseased tissue into healthy plants. At present no information is available regarding the possible role of insects in spreading purple dwarf. Apparently the disease must be spread by some agent to a few plants each year, and definite symptoms fail to appear immediately in such cases, or it develops in some obscure manner in the tubers subsequent to their harvest. Possibly contact between the foliage of purple dwarf and healthy plants in the field would be sufficient to transfer the malady, but no evidence of this has been observed to date. At any rate, plants that develop definite purple dwarf symptoms from diseased tubers either die before new tubers are formed, or, if any develop, they are small, severely netted, and unable to produce sprouts that emerge above ground. This may explain why the malady does not increase more rapidly. It is hoped that results obtained from grafting diseased tissue into healthy plants will throw necessary light on some of the obscure points mentioned.

Discussion

Although many aspects of the purple dwarf disease remain to be clarified, the more important distinguishing characteristics of the malady can be presented at this time.

In several ways, purple dwarf seems to be essentially different from yellow dwarf (Barrus and Chupp (2), Muncie (3), and Walker and Larson (5)). For example, there is no prompt die-back from the tips of affected plants.

Also, the tubers are uniformly small, or do not develop, and they are not misshapen. Affected plants die rather slowly and do not wilt suddenly. Further, pith necrosis is by no means a diagnostic feature of purple dwarf, because very often it is not present in the stem, and apparently absent in the tuber. Moreover, internal net necrosis in purple dwarf tubers is a typical symptom that is confined to the phloem throughout the tuber and does not consist of other groups of disorganized cells. Finally, the foliage of affected plants develops typical purplish pigments, seldom yellowish ones.

On the other hand, purple dwarf and yellow dwarf are similar in certain respects, because both diseases are apparently of a virus nature and carried by the tubers. Moreover, if healthy plants become infected later during the growing season, they apparently fail to develop easily recognizable purple dwarf symptoms either on the foliage or in the tubers. Further research will show whether this can be proved to be the case.

Purple dwarf also appears to be different from the blue stem disease described by Orton and Hill (4). In contrast with purple dwarf, definite symptoms of blue stem apparently develop quickly on normal plants at any stage from the beginning of tuberization to maturity, and the plants usually wilt within two weeks. Another important difference is that the blue stem disease is not perpetuated through the tubers, and that tubers of marketable size may be produced if the infection is not too early. Further, infection of plants under field conditions seems to be general and the blue stem symptoms unmistakable. Finally, the dendritic necrosis seems usually confined to the first half or quarter of the stem end of the tuber, but in purple dwarf it is characteristic for this necrosis to permeate the entire length of the tuber.

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STUDIES ON THE PERENNIAL RUST *PUCCINIA MINUSSENSIS*[1]

By A. M. Brown[2]

Abstract

The permanent infection of rust-free *Lactuca pulchella* plants by *Puccinia minussensis* may be initiated by inoculating the primary leaves of seedlings, or the rhizome buds of older plants, with either aeciospores or urediospores, or by packing teliospores into the leaf axils of mature plants and subsequently using the infected nodal portions as propagation cuttings. When a primary leaf of a seedling becomes infected, the binucleate mycelium progresses along the midrib and petiole to the stem and advances down it into the rhizomes but, before reaching the terminal buds of the rhizomes, its nuclear condition changes, as only uninucleate mycelium has been observed in the bud scales. Infected rhizome buds develop into young shoots that usually first produce pycnia, although, in cases of delayed mycelial development, telia appear on the more mature plant followed by pycnia on subsequent new axillary growth. The perennial mycelium seems to alternate from the binucleate to the uninucleate condition and vice versa, depending apparently on the maturity of the host tissue involved and the food supply available to the fungus.

Introduction

In the past, perennial rusts of plants have attracted the attention of several investigators. Plowright (6) appears to have been the first to successfully initiate permanent infection in a host plant. On sowing teliospores of *Endophyllum Euphorbiae* DC. on very young plants of *Euphorbia amygdaloides*, he noted that the subsequent infection progressed along the leaf petioles to the stems, where the mycelium could be found "in the pith and inner bark." The mycelium then invaded the subterranean part of the host. Describing further development, he states: "The foliage and shoots sent up by it in the following year, are pervaded by the perennial mycelium, and produce aecia abundantly during the spring, but the late summer and autumn foliage are somewhat shorter. The next vernal foliage is, however, aecidiiferous." Plowright (6) failed, however, to secure the permanent infection of old plants, apparently because defoliation occurred before the mycelium reached the stems.

Dodge (2), in his investigations on *Gymnoconia peckiana* (Howe) Trotter and *Kunkelia nitens* (Schw.) Arth., succeeded in permanently infecting *Rubus* species by inoculating emerging shoots. Older shoots, however, when inoculated near the top, always failed to become permanently infected.

From a cytological study of three rusts, all of which he regarded as perennial, Olive (5) concluded that the uninucleate mycelium and the binucleate mycelium of these rusts were of independent origin. Kursanov (3), in his

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interpretation of the nuclear behaviour of *Trachyspora Alchemillae* Fckl., seems to have accepted Olive's explanation, whereas Lindfors (4) concluded that all the mycelium of this rust was uninucleate and that binucleate cells arose as a result of cell fusion or nuclear migration.

Among the perennial rusts found in Western Canada is *Puccinia minussensis* Thum., which occurs abundantly on *Lactuca pulchella* (Pursh) DC. This rust is euautoecious. The aecia are reported to occur on a perennial mycelium (1), the uredia and telia presumably arising as a result of secondary infections. Field observations made at Winnipeg by the writer indicated, however, that uredia and telia might also arise from perennial mycelium, and this observation led to the initiation of experiments dealing with the life cycle and nuclear behaviour of this rust.

Experimental Procedure and Results

Early in June, 1932, naturally infected plants of *L. pulchella* were transplanted from the field to the greenhouse. At this time pycnia had developed on the leaves and the infected plants gave off a sweetish odour that was noticeable, especially in damp weather, at a distance of several feet. As the pycnium-bearing leaves aged, aecia developed along the midribs, forming progressively from the petioles to the tips of the leaves. Unlike the pycnia, which covered the whole leaf surface, the aecia were largely confined to the midribs, although both pycnia and aecia occurred sparingly on the stems. In several aecia, the aeciospores were suppressed and teliospores developed in their place (Fig. 1), whereas, on some leaves, telia followed pycnia directly (Fig. 2). In the latter case, the telia appeared on the leaf blades and were not confined to any particular location, as the aecia had been. Sometimes uredia, interspersed among the aecia, appeared along the midribs, but urediospores did not displace the aeciospores as did the teliospores just mentioned.

When the infected plants died back in October, the rhizomes were removed from them and reset singly in pots. In January, 1933, new shoots emerged in these pots and a majority of them, when from three to four inches high, produced pycnia and then aecia in due course. On some of the shoots developing pycnia, the short-cycling tendencies referred to above were encountered. On two of the plants arising from the reset rhizomes, however, pycnia did not develop but, when the plants reached maturity, telia were produced on the lower half of the stems. These stems were then cut back, leaving only the parts on which the telial lesions were present. Three or four weeks later, axillary growth developed and on this new growth pycnia and aecia developed in sequence.

The results secured in the experiments just described indicated that the perennial mycelium changed from the uninucleate to the binucleate condition and vice versa, within the host. In order to examine this nuclear phenomenon more thoroughly, a series of experiments were initiated.

PLATE I

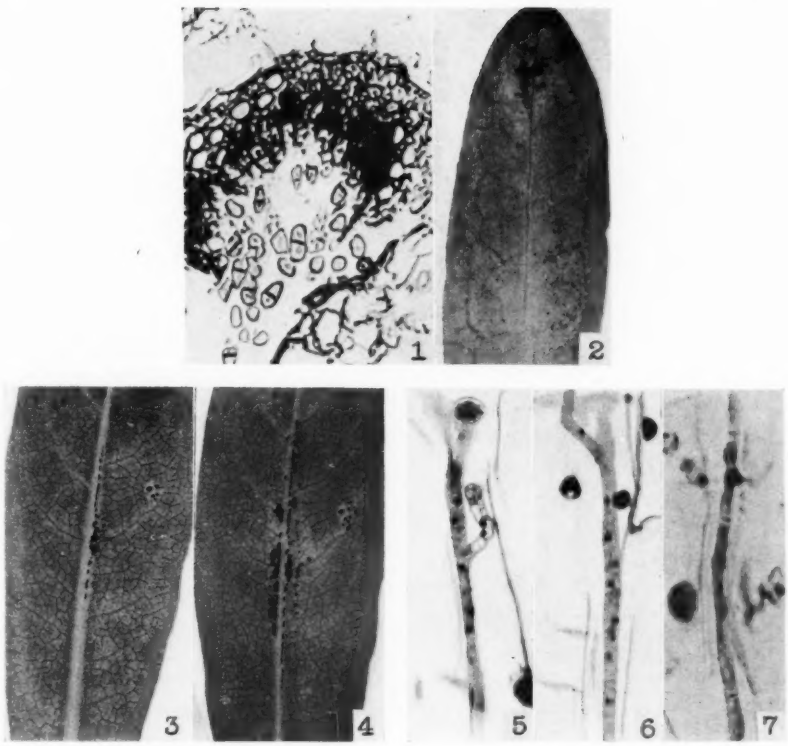

FIG. 1. *Photomicrograph of a section of an aecium cup. Teliospores are present instead of aeciospores.* × *350.*

FIG. 2. *An infection in which telia occurred directly after pycnia. The pycnia are still seen following the leaf veins. About natural size.*

FIG. 3. *A uredial infection on Lactuca pulchella. After invading the midrib, it is extending along it in both directions.*

FIG. 4. *The same infection 28 days later. Compare rate of growth with that of the localized infection of the same age on the right.*

FIG. 5. *Photomicrograph showing paired and single nuclei in the mycelium of a rhizome, section cut from the area adjacent to the root crown.* × 500.

FIG. 6. *Photomicrograph showing mostly single nuclei in a section cut from a rhizome near the terminal bud.* × *600.*

FIG. 7. *Photomicrograph showing uninucleate mycelium in the scales from the terminal bud of the rhizome.* × *900.*

Inoculation of Seedlings

Ten seedling *L. pulchella* plants grown in the greenhouse were inoculated in August, 1933, by sowing urediospores on the primary leaves, so that the resulting infections would occur on or near the leaf midribs and adjacent to the petioles. Two weeks later several isolated uredial infections developed close to the midribs and eventually invaded them. The uredial infections after invading the midribs rapidly expanded and in due time reached the stems by way of the petioles. One of these infections was closely observed and was twice photographed. At the time of the first photograph (Fig. 3) it was 25 mm. long; at the time of the second (Fig. 4), 28 days later, it measured 45 mm. Its growth rate, which was therefore 0.72 mm. in each 24 hr., can be compared to that of the isolated infection of the same age occurring on the right of the midrib.

The leaves on which midrib infections developed were kept in a vigorous state of growth by pinching out the terminal bud of the plants bearing them. Late in December, when all the midrib infections had invaded, or almost invaded, the stems, the hosts were placed beneath a bench in the greenhouse where they remained until February, 1934. During this time the temperature of the greenhouse was maintained close to 13° C. About the end of February the pots were taken from this greenhouse. A large proportion of the original stems in each pot remained green. Two of the pots were placed in a warm greenhouse. From the remaining eight pots the rhizomes were removed and reset singly, the stems and other plant parts being discarded.

In March, new axillary growth appeared on the stems in the two undisturbed pots, and this new growth produced, for the most part, pycnia and aecia in sequence. Occasionally teliospores appeared in place of aeciospores in some aecia, or telia occurred directly after pycnia. From the reset rhizomes new shoots emerged in May, and a majority of them produced pycnia. On one shoot, however, telia developed directly after pycnia, and, on another shoot, uredia occurred, interspersed with aecia. Two shoots arising from reset rhizomes continued without visible rust infection until they approached maturity; then telia developed on the lower half of the stems. The stems were then cut back to a point just above the uppermost telial sorus. This induced new axillary growth on which pycnia and aecia were produced in sequence, but with teliospores instead of aeciospores in some aecial cups.

From these results it seemed clear that the perennial mycelium of *Puccinia minussensis* could, under certain conditions, be initiated by urediospore inoculation of its host. The initial mycelium of the infection would therefore be binucleate; but, to produce pycnia in the new axillary growth of the host, the binucleate condition of the mycelium must have undergone change. Apparently, however, the change from the binucleate to the uninucleate condition did not always occur in every plant, as telia, unaccompanied by pycnia or aecia, developed on the new growth of several plants.

The unusual nuclear behaviour just mentioned was investigated more fully by repeating the experiments described above during the years 1934 to 1937. During this period, 50 seedling plants were inoculated with aeciospores so that the ensuing infections would readily invade the stems by way of the midribs and petioles. The inoculated plants were treated in a manner similar to that adopted in the previously described experiments, and the results secured were identical with those already given. In addition, 10 uninoculated plants were maintained as checks, but they remained free from infection. Moreover, repeated attempts to establish the systemic mycelium of this rust in maturing blue lettuce plants by aeciospore or urediospore inoculation failed, owing probably to defoliation before the infections could invade the midribs, petioles, and stems.

Inoculation of Rhizome Buds

An examination of the rhizomes of older plants showed that well developed stomata were present on the scales of the terminal buds. As the bud scales could be separated without causing injury, aeciospores were inserted between the scales of 50 buds on the rhizomes of rust-free plants. Ten uninoculated buds on other rhizomes were kept as checks. The rhizomes bearing the inoculated buds were kept in a moist chamber for two days before they were reset singly in pots. The controls were treated similarly. Thereafter the pots were kept under ordinary greenhouse conditions. Two months later new shoots appeared above ground in all the pots; when they were from two to three inches in height, 47 out of 50 of the shoots originating from inoculated buds produced pycnia whereas the shoots from the uninoculated buds remained rust-free.

As a rule, aecia followed pycnia, but, as in the former experiments, teliospores sometimes occurred instead of aeciospores, and uredia appeared in association with aecia. However, three shoots from inoculated buds continued without visible rust infection until they were almost mature plants; at that time telia occurred on the lower half of the stems. As in the former experiments, the stems were cut back to just above the telial sori; on subsequent axillary growth were produced pycnia and aecia, as had happened in the previous experiment.

From these results it is seen that the inoculation of rhizome buds with aeciospores gives results identical with those obtained by inoculating leaves of seedling plants with aeciospores or urediospores. In passing, it may be observed that by inoculating the rhizome buds the time of experimentation was reduced by approximately one-half, as pycnia developed within two months, whereas, with seedling inoculation, pycnia developed after four months.

Inoculation of Mature Plants

In these experiments, many attempts were made to establish systemic infection by inoculating mature plants with aeciospores or urediospores, but always with negative results. This was the experience of Plowright (6) with *Endophyllum Euphorbiae* and Dodge (2) with *Gymnoconia peckiana* and

Kunkelia nitens. Positive results, however, were eventually obtained by one method. Teliospores were packed into the leaf axils of plants almost mature. Two weeks later, wart-like swellings appeared on the stems just above the nodes, where teliospores had been in contact with them, and on these swellings telial sori developed. By wrapping the infected areas in moist cotton wool, adventitious roots were induced to develop at the nodes, and, from such nodes, cuttings were obtained that eventually gave rise to permanently infected plants. Just how the infections were brought about is not known, but it is presumed that sporidia from germinating teliospores initiated them. The variations previously encountered in the life cycle of the organism were also observed in this experiment.

Nuclear Behaviour

An examination of plants in which permanent infection was induced through inoculation of the leaves by aeciospores or urediospores, showed that the binucleate mycelium invaded the stems from the petioles and that the mycelium remained binucleate until it invaded the root crown. From there the mycelium penetrated the rhizomes and, in them, evidently changed to the uninucleate condition. At any rate, microtome sections cut from rhizomes near the root crown showed large mycelial cells, each containing several paired and a few single nuclei (Fig. 5). Between this area and the terminal bud of the rhizome each mycelial cell, still large, contained few paired and many single nuclei (Fig. 6), but, in the bud scales, only uninucleate cells were found (Fig. 7).

A similar nuclear condition was observed in sections cut from axillary shoots arising from stems that had produced only telia and had been cut back to induce new growth. On the new shoot, pycnia first appeared on the second pair of new leaves as they unfolded. The mycelium in the basal tissue of the shoot was binucleate but uninucleate in the terminal bud, and obviously so in the leaves producing pycnia. A change in the nuclear condition of the mycelium must therefore have occurred in the lower part of the shoot. The aecial primordium was uninucleate. The return from the uninucleate to the binucleate condition occurred at the base of the aecium.

From the experimental results, it appears evident that the nuclear condition of the mycelium is closely related to the age of the tissue in which it is present and is apparently dependent, as suggested by Whetzel, Jackson, and Mains (7), on the maturity of the host tissue and the supply of food available to the fungus.

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THE "LEAF GAP" IN *EQUISETUM*[1]

By Ruth Moore[2]

Abstract

A previously undescribed feature of the structure of the stem in *Equisetum* has been found. This conforms more nearly with a true leaf gap than any other feature of *Equisetum* hitherto so considered. Its interpretation and bearing on the classification of the vascular plants into Lycopsida and Pteropsida are discussed.

Introduction

In 1899, Jeffrey (4) proposed a division of the vascular plants into two groups, the Lycopsida and the Pteropsida. The Lycopsida comprised the vascular forms below the ferns and thus included the genus *Equisetum*. Its members were characterized, among other features, as microphyllous plants that were without foliar gaps. The Pteropsida, on the other hand, were considered fundamentally megaphyllous, with a well developed parenchymatous leaf gap in the cauline vascular tissue immediately above the outgoing leaf trace, leaving the parenchyma of the medulla and cortex in direct connection with each other. The classification was for a time widely accepted and the belief is still general that a leaf gap is not present in *Equisetum* (3, p. 99) despite the fact that certain features in the anatomy of the plant have been held by various writers to represent this gap. For example, in the stem the large parenchyma lacunae between the forking vascular strands have been interpreted as foliar gaps. However, since vascular tissue of the stem usually intervenes between trace and lacuna, and since in those forms that have a continuous endodermis, this endodermis remains unbroken, the lacunae do not fulfil one of the basic requirements of a leaf gap in that they are not in direct contact with the leaf trace and they do not place the pith and cortex in connection. In the cone, too, Browne (1) has suggested that the parenchymatous strands between the conducting tissues of the axis are leaf gaps, but here also nodal xylem may separate trace and parenchyma. Cases where this is not true and the parenchyma lies immediately above the sporangiophore trace could be explained not as a primary, but as a secondary feature resulting from reduced xylem development. Moreover, as in the stem, the endodermis, in certain forms at least, separates the medulla and cortex. Johnson (5) appears to be the only one to recognize the importance of this feature. In his study of the cones of *E. scirpoides*, he observed the reduction in supranodal wood bringing the trace and the parenchyma together and mentioned the fact that the presence of the unbroken endodermis across the gap was, in his opinion, the only indication that it was not a true foliar gap. In addition, these parenchyma strands are probably homologous with the lacunae of

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Contribution from the Department of Botany, University of Toronto, with assistance from the National Research Council of Canada.

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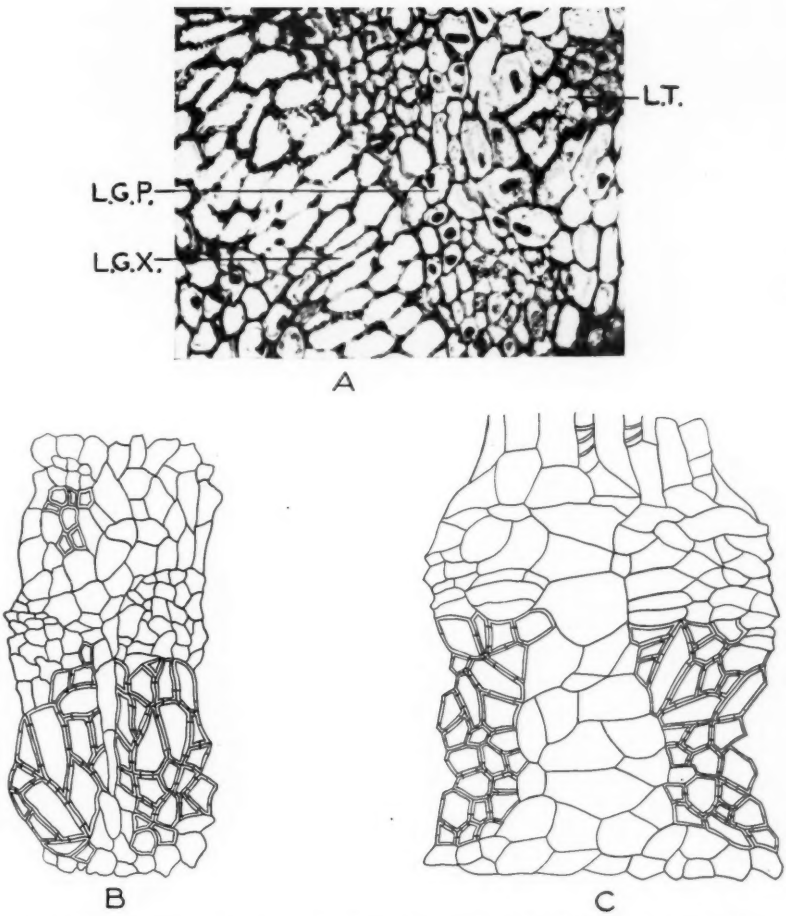

FIG. 1. *Transverse sections of the "leaf gap" in the stem of Equisetum. A: E. sylvaticum; L.G.P., "leaf gap" in the phloem; L.G.X., "leaf gap" in the xylem; L.T., leaf trace. ×280. B: Vegetative stem of E. arvense. ×300. C: Fertile stem of E. arvense. ×300.*

the stem which, as previously noted, cannot be considered foliar gaps. Of course it might be argued by those who believe the cone to be a conservative region that the apparent foliar gap that may occur there when the xylem above the trace is undeveloped is a primary character and that the supranodal wood intervening between the trace and the parenchyma in both stem and cone is a later development obscuring the true nature of the parenchyma as a foliar gap. However, another feature found by the writer in *Equisetum*, may correspond more completely with the Pteropsid leaf gap.

Description

Transverse sections cut through the node of the *Equisetum* stem show frequently, although by no means always, a band of parenchyma one to two or three cells wide, and as many high, crossing the conducting tissue immediately above the departing leaf trace (Fig. 1A). This band is closed over by vascular elements that separate it from the large lacuna above. It is not illustrated here in longitudinal section, but it may be described as lying above any of the leaf traces of Fig. 2C, very similar, except for its smaller development, to the parenchyma island associated with the trace at the extreme right of Fig. 2B. In some cases the pith is in direct contact with the parenchyma of the cortex through the narrow band; in others, the endodermis is continuous so that no actual connection is made. In those species in which it is present the internal endodermis is always unbroken. The parenchymatous band has been observed in seven species examined:— *E. arvense* L., *E. giganteum* L., *E. limosum* L., *E. palustre* L., *E. praealtum* Raf., *E. scirpoides* Michx., and *E. sylvaticum* L. It is particularly well marked in the vegetative nodes of the fertile stem of *E. arvense* (Fig. 1C) where, in contrast with the sterile stem (Fig. 1B), the parenchmya cells are somewhat more numerous and are not radially elongated. In *E. palustre* and *E. scirpoides*, both of which are species with somewhat reduced xylem, the nodal xylem may occasionally fail to close over the gap whereas in *E. praealtum*, sometimes also in *E. arvense*, the number of parenchyma cells may be increased so that the band extends down the sides and underneath the leaf trace. Apparently a somewhat similar condition has been observed by Browne (2) in *E. kansanum* where she notes that the leaf trace passes through a "parenchymatous gully". Further study of the extent of these variations in the different species would be necessary to determine whether they have taxonomic significance.

Cones of six of the above mentioned species were also studied. No evidence of a gap was found at any of the fertile nodes. This was true of both normal and markedly abnormal cones. Only in the case of abnormal and infertile annuli that were leaf-like and vascularized were there any indications of its presence.

Discussion

It appears that the only previous reference made to this parenchyma band is Browne's mention of the parenchyma gully in *E. kansanum*. The band differs from the commonly accepted type of Pteropsid leaf gap in its very

small size, in the irregularity of its occurrence and its form, and in the frequent presence of the unbroken endodermis. However, as a parenchyma band passing through cauline vascular tissue directly above a leaf trace, at times connecting pith and cortex, it appears to justify consideration as a leaf gap, in which light it is here proposed to discuss it. The only other interpretation that seems possible is that it might be storage parenchyma associated with the assimilatory function of the leaf; in *Equisetum*, however, the leaf is of minor importance as a photosynthetic organ. In the fertile shoot of *E. arvense* (which lacks chlorenchyma tissue) leaf base storage would be very unlikely, but here the gap is particularly well developed. In addition, this fertile stem has little need for stored food since, formed underground and dependent on subterranean organs for nourishment, it shoots up with rapid cell expansion and after a brief period of reproductive activity dies away.

The irregularity in occurrence and form of the "leaf gap" suggesting that it is not a well established character of the plant, raises the question as to whether it is to be regarded as a comparatively recent acquisition in the genus or as an ancient feature that is gradually disappearing. That it may be a rather new development might be concluded from its exceptionally strong formation in the fertile stem of *E. arvense*, which, if Schaffner's (7) view be accepted, is a phylogenetically recent organ. The fertile stem also is provided

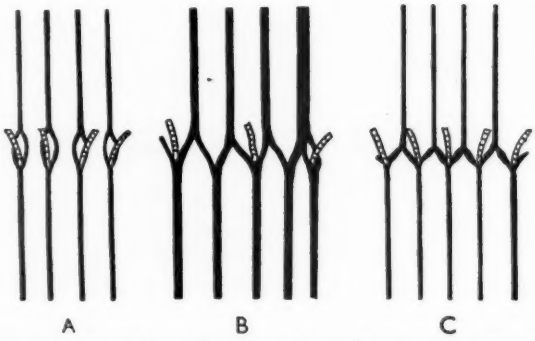

FIG. 2. *(From Walton (8)). The relation between internodal bundles (solid) and leaf traces (hatched). A: Asterocalamites. B: Calamites. C: Equisetum.*

with a large leaf sheath; this might be expected to involve the presence of a gap since the passage outward through the axial stele of a considerable mass of conducting tissue, such as the trace supplying a large leaf, should leave behind a gap in the main stele. On the other hand, a suggestion that the gap may be not recent, but long established, is found in the fact that, in an early stage of the development of the nodal region, the young stem bundle as figured for *E. arvense* by Queva (6) is cut into two similar halves by a wide parenchyma band, the result of the leaf trace having carried out the central part of the bundle. Moreover, a comparison of *Equisetum* with the

Devonian fossil *Asterocalamites*, to which *Equisetum* is doubtless related, gives further indication that the gap may not be recent. *Asterocalamites* had large leaves and if a gap accompanied these leaves and was not associated with leaf base storage, it would be an indication either of a true megaphyllous organization or of an enation type of leaf that had attained sufficient size for its trace to leave a gap in the vascular tissue of the stem. A diagram in a textbook by Walton (8, p. 80) showing the course of the vascular strands in *Asterocalamites* suggests that a leaf gap did occur in this genus (Fig. 2A). The arrangement of the cauline vascular tissue differs from that found in *Equisetum* (Fig. 2C) in that the longitudinal bundles did not fork at the nodes; the result was, therefore, that the leaves, not the branches, alternated with the parenchyma strands of the stem. If, in the course of evolution, there was a gradual shift from one condition to the other, it would not be difficult to imagine that a mass of parenchyma such as that figured by Walton as enclosed by the vascular loop above the departing leaf trace might have become reduced to the narrow parenchyma band that may occur to-day above the leaf trace of *Equisetum*. However, even the acceptance of such an homology does not decide the interpretation of the *Equisetum* "leaf gap" since no reference is made to the *Asterocalamites* "gap" by Walton in the text, and it cannot be determined from the diagram alone whether it is to be considered a true foliar gap.

That the absence of the "gap" from the cone in any way affects the question of a *leaf* gap in the plant depends on the consideration of the sporangiophore as a primarily foliar organ, an interpretation that is by no means universally accepted. To those who favour the view that the sporangiophore is of a foliar nature its absence would be an indication of the relatively recent appearance of the gap since without doubt the lines of evolution of stem and cone have been divergent, whether the cone is looked upon as a primitive region retaining ancestral features or as a specialized region developing characters distinct from those of the vegetative stem.

There remains the question of the bearing of these observations on Jeffrey's Lycopsid-Pteropsid classification. Since the classification was presented, there have been various proposals to transfer the Equisetales, a family comprising *Equisetum* and its fossil allies, to a separate group, the Sphenopsida (Articulatae). Two closely related fossil families were also to be placed in this group that was to rank equally with the Lycopsida and the Pteropsida. In this connection, various lines of evidence have been brought forward, particularly by fossil botanists who emphasize the verticillate organization of the Sphenopsids. Still further evidence for the separation of the Equisetales from the Lycopsids is offered by the presence, in at least the living genus of the family, of the peculiar type of "leaf gap" described above, no matter what interpretation is placed on this structure.

In conclusion it should be noted that before there can be a satisfactory understanding of the meaning of the "leaf gap" development in *Equisetum*,

a further detailed investigation of the nodal anatomy of the genus *Asterocalamites* must be made with a view to determining whether a true leaf gap occurred in this large-leaved primitive form.

Acknowledgments

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CANADIAN WILTSHIRE BACON

XVI. COLOUR AND COLOUR STABILITY OF PORK AFTER FROZEN STORAGE AND CONVERSION TO BACON[1]

BY W. H. COOK[2]

Abstract

Temperatures below −17.7° C. (0° F.) and moisture resistant, preferably impermeable, wrappings are desirable for the retention of colour of pork during storage. Although pork thawed in air or water was lighter in colour than that thawed in brine or pickle, the difference was markedly reduced after curing and maturation. Nevertheless, the colour of the final product was significantly correlated with that of the pork after thawing. Samples stored under conditions that retain the original colour, or subsequently thawed by procedures that produce light colours, are the least stable after defrosting. On the average there was comparatively little difference between the colour of exposed and internal samples, although the former were more variable. Pale-drying yielded samples with a lighter internal colour than did the usual smoking procedure.

Introduction

In Canada it is frequently necessary to store appreciable quantities of frozen pork for subsequent use in the manufacture of bacon. Storage conditions that yield a product suitable for immediate consumption as pork may not be suitable for the production of bacon of acceptable quality, since certain changes that occur to only a minor extent during storage may be exaggerated during thawing, curing, or maturing. In a previous paper (2) on the frozen storage of pork, it was shown that storage temperature had a significant effect on the colour of the lean and the rancidity in the fat. Nevertheless, within the temperature range commonly employed for the storage of pork for comparable periods in commercial practice (−15° C. or lower) these changes were too small to have any serious effect on the quality of the pork for immediate consumption. The investigation reported here was undertaken to determine the effects of various storage conditions and thawing procedures on the quality of the product at all stages of processing, including the matured bacon.

Colour and colour stability of the lean, and the peroxide oxygen and free fatty acid content of the fats, were used as criteria of quality, since changes in bloom caused by drying, and changes in the pigments and fats due to oxidation are among the first storage defects to become evident. The results of the fat analyses will be reserved for a later paper.

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Material and Methods

The material, which consisted of full-length rib-in pork backs, was subjected to various storage conditions. Duplicate samples were allotted at random to the various sets of conditions, giving a total of 30 pieces. The storage temperatures were −6.6°, −12.2°, −17.7°, −23.4°, and −29.0° C. (20°, 10°, 0°, −10°, and −20° F.). Since it was difficult to provide precise control of relative humidity for these large cuts, three wrapping practices, with wrappers graded to represent various degrees of permeability to water vapour, were substituted for controlled humidities at each storage temperature. This procedure emulates commercial practice where wrapping is used to reduce surface drying. The wrappings used were as follows: single layer of waxed glassine paper, adequately overwrapped and tied but not sealed; two layers of waxed glassine, each layer applied and tied separately as above, and not sealed; and aluminium foil solidly laminated to sulphite paper and sealed. All the packages were protected against mechanical damage during storage by overwrapping with a single layer of heavy Kraft paper.

TABLE I

TREATMENT OF SAMPLES AFTER REMOVAL FROM STORAGE

Stage	Method	Temperature, °C.	Time, hr.	Additional exposure after sampling
Thawing	In air (wrapped)	10.0	25	6 hr. in air at 7.5° C.
	In brine	4.5	5	2 hr. in air at 7.5° C.
	In pickle	4.5	4½	2 hr. in air at 7.5° C.
	In water	38.0	1⅓	1¼ hr. in air at 7.5° C.
Cure	Wiltshire pickle	3.6	88	Sampled on removal
Maturation	In air (covered)	3.2	168	Sampled on removal
Subsequent treatment	Smoked in commercial smoke house	55	7¼	Sampled for colour on following day, and for fat analyses five days later. Temp. 10° C.
	Heated in laboratory oven	55	7¼	

After 48 weeks' storage under these conditions, the material was examined, and each piece cut into two approximately equal portions. The four pieces thus obtained from each storage treatment were allotted at random to the four thawing procedures described in Table I. The exposure period was that required to bring the internal temperature of the samples to a curing temperature of 3.6° C. (38° F.). The brine and pickle were maintained at the specified temperatures with electrical heaters, and the water by the addition of steam.

All samples were cured together in a commercial Wiltshire pickle (Table I), obtained from a packing plant. Pumping was considered unnecessary for these comparatively small pieces, and the curing period was reduced to less

than four days in an attempt to approximate the salt content obtained commercially. Analysis of four samples, taken at random from each of the four groups of thawing procedures, after curing and smoking or pale-drying, showed average chloride, nitrate, and nitrite contents of 5.93, 0.039, and 0.003%, respectively. Those thawed in pickle had a somewhat higher chloride content (6.57%) than those thawed by the other procedures, but there were no other systematic differences. These values are well within the range of those obtained in commercially cured and smoked samples (1, 5).

The material was sampled after thawing, cure, and again after a 7-day maturation period. This was the last systematic sampling that represented all storage treatments and thawing procedures. The 60 pieces remaining as residues were then divided at random into two groups of 30; one group was smoked in a commercial smokehouse, and the other pale-dried by heating at the same temperature under similar temperature conditions for a comparable period in a large laboratory oven. Measurements were made on these two groups to compare the effect of smoking and pale-drying.

The colour measurements were made by a procedure already described (3). Colour stability was estimated by repeating the colour measurements after the samples had been exposed for 72 hr. at 10° C. and 95% relative humidity.

Colour Measurements on Internal Surfaces

In order to reduce the data, only the average values for each condition and an analysis of variance on the detailed results are reported (Tables II and III). It is evident from the latter that each of the four primary factors (storage temperature, wrapping, thawing, and stage of conversion) had significant effects on each of the colour components. In addition the factors frequently showed significant differential effects. Since the direct effect of a given condition seldom significantly exceeded the differential effects, the tests of statistical significance were made by comparison with the residual variance or experimental error.

Since the scatter of the several colour components are correlated (4) only the total of all scatters, or brightness, will be discussed. The colours of the samples stored at −6.6° and −29° C. were significantly brighter than those stored at the intermediate temperatures. The increased brightness at −6.6° C. is due to methaemoglobin formation, whereas that observed at −29° C. approximates the value for fresh pork (2). Since the brightness at the three intermediate temperatures was below normal it is concluded that some drying occurred. At −12.2° C. this was possibly offset by slight methaemoglobin formation, although no visible effects were evident.

The differences observed between the various wrapping procedures were as expected. The brightness increased significantly in order of increasing impermeability of the wrapper to water vapour, showing the effect of differential evaporation from the stored product.

TABLE II

COLOUR OF PORK STORED AND CURED UNDER VARIOUS CONDITIONS

Colour component	Average scatter at each storage temperature					
	−6.6° C.	−12.2° C.	−17.7° C.	−23.4° C.	−29.0° C.	Necessary difference
Blue	12.9	11.6	11.8	11.2	12.2	0.70
Green	17.0	15.1	15.2	14.5	16.1	0.96
Red	27.4	24.8	24.5	23.8	26.2	1.21
Total (Brightness)	57.2	51.5	51.4	49.6	54.4	2.77

	Average scatter for each method of wrapping			
	Single wrap, waxed paper	Double wrap, waxed paper	Single wrap, aluminium foil	Necessary difference
Blue	11.3	12.0	12.5	0.16
Green	14.6	15.6	16.5	0.74
Red	24.2	25.0	26.9	0.94
Total (Brightness)	50.0	52.6	56.0	2.15

	Average scatter for each method of thawing				
	In brine	In pickle	In air	In water	Necessary difference
Blue	11.4	11.6	12.2	12.5	0.59
Green	14.6	14.8	16.1	16.6	0.86
Red	23.8	23.8	26.6	27.1	1.08
Total (Brightness)	49.8	50.3	55.0	56.2	2.48

	Average scatter at each stage of conversion			
	After defrosting	After curing	After maturation	Necessary difference
Blue	13.3	10.8	11.7	0.16
Green	18.3	13.6	14.7	0.74
Red	28.3	23.6	24.2	0.94
Total (Brightness)	60.0	48.0	50.6	2.15

The thawing procedures fell into two groups with respect to their effect on colour. Immersion in brine or pickle produced samples that did not differ significantly from one another, but both yielded pork considerably darker in colour than that thawed in air or water. An adequate explanation for this behaviour cannot be given, but the withdrawal of water from the pork immersed in the salt solutions may be the responsible factor. A similar behaviour was observed during cure when all of the samples, regardless of the previous storage and defrosting treatment, darkened considerably. During subsequent maturation there was a comparatively small, but significant increase in brightness.

Since all of the primary factors affected the colour, differential effects were to be expected over the range of each condition studied. The analysis of variance shows that most of these interactions were significant, and of a magnitude comparable to the primary factors. Thus, on the average, wrap-

TABLE III

ANALYSIS OF VARIANCE OF COLOUR MEASUREMENTS

Variance due to:	D.f.	Mean square			
		Blue	Green	Red	Total (Brightness)
Temperature	4	13.9***	34.4***	74.8***	328 ***
Wrapping	2	23.2***	57.8***	122 ***	539 ***
Thawing	3	12.3***	43.9***	140 ***	476 ***
Stage of conversion	2	101 ***	367 ***	387 ***	2376 ***
Temperature × wrapping	8	13.6***	30.9***	53.4***	273 ***
Temperature × thawing	12	6.4***	17.1***	27.2***	140 ***
Temperature × stage	8	1.4	1.3	0.8	9.0
Wrapping × thawing	6	5.1*	12.0*	21.3**	96.8*
Wrapping × stage	4	0.8	1.3	1.2	9.1
Thawing × stage	6	13.3***	42.3***	149 ***	489 ***
Error	124	2.0	4.2	6.7	35.2

* *Indicates 5% level of significance compared with residual.*
** *Indicates 1% level of significance compared with residual.*
*** *Indicates 0.1% level of significance compared with residual.*

ping had significant effects, but the differences were smaller at low, than at high, storage temperatures. Similarly, pork stored under conditions that may tend toward the production of a lighter or darker colour may be affected differentially by the thawing procedure, those thawed in air or water being lighter than those immersed in brine or pickle. In this connection it is of interest to note that the stage of conversion of pork to bacon did not show differential effects with any of the factors except method of thawing. This implies that certain thawing procedures affect colour in the same manner as curing, whereas others do not. It is therefore of interest to examine the effects of defrosting and stage of conversion in greater detail.

Table IV shows the brightness at each stage for each method of defrosting, averaged for all storage treatments. Immediately after thawing in brine, the value of the colour is approximately the average for pork. By comparison, pickle causes slight darkening whereas, at this stage, the other two methods of thawing yield much lighter samples. During cure all of the samples darkened somewhat, the most marked change occurring in the bright samples that had been thawed in air or water. All have about the same brightness after cure. During maturation all samples became brighter, with those thawed in pickle showing the greatest increase.

The extent of correlation of the colours observed at the three stages was determined by computing simple coefficients of correlation. The coefficients of correlation between the brightness before and after cure were generally largest, and those between the values before and after maturation lowest (Table IV). These lower values are doubtless attributable to the smaller average change during maturation that rendered the experimental error

TABLE IV

EFFECT OF METHOD OF THAWING ON COLOUR (BRIGHTNESS) OF LEAN AT VARIOUS STAGES DURING CURE

Thawed in	Total scatter, %		
	After thawing	After cure	After maturation
Brine	53.8	46.7	48.9
Pickle	49.9	47.5	53.7
Air	67.0	48.6	49.3
Water	69.1	49.2	50.4

	Coefficients of correlation between total scatter at the various stages			
	Degrees freedom	After defrosting with after cure	After cure with after maturation	After defrosting with after maturation
Brine	13	0.84**	0.54*	0.64**
Pickle	13	0.91**	0.78**	0.76**
Air	13	0.65**	0.56*	0.64**
Water	13	0.70**	0.52*	0.57*
For all methods	58	0.63**	0.60**	0.37**

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

proportionately larger. The coefficients given in the last column of the table are of greatest interest since they show the extent to which the colour of the bacon depends on the colour of the thawed pork. These are of a magnitude sufficient to indicate a considerable degree of dependence, being greatest for material thawed in pickle (2).

Colour Stability Measurements on Internal Surfaces

The results of the colour stability measurements appear in Tables V and VI. A number of changes in the individual components did not attain significance presumably owing to the proportionately greater experimental error (3). Brightness stability, the only attribute of colour quality discussed here was usually affected significantly by each of the four factors under investigation. The effect of storage temperature and wrapping procedure, however, had less effect than the method of thawing and the stage of conversion. The effect of storage temperature and wrapping can be summarized by stating that a low storage temperature and wrapping in an impermeable wrapper, conditions that favour maintenance of the original colour during storage, yield material that suffered the most darkening during exposure. Reasons for this behaviour have already been given (12).

The effect of the method of thawing shows a similar behaviour. The lighter samples produced by thawing in air and water were least stable during exposure whereas the dark samples produced by thawing in brine and pickle showed little change. On the average, all samples darkened after thawing; after

TABLE V

COLOUR STABILITY OF PORK STORED AND CURED UNDER VARIOUS CONDITIONS

Colour component	Average change in scatter at each storage temperature					
	−6.6° C.	−12.2° C.	−17.7° C.	−23.4° C.	−29.0° C.	Necessary difference
Blue	+0.022	+0.111	−0.044	−0.183	−0.200	0.27
Green	−0.011	+0.211	+0.222	−0.092	−0.258	0.34
Red	−0.844	−0.964	−0.992	−1.04	−1.25	0.55
Total (Brightness)	−0.830	−0.647	−0.814	−1.32	−1.71	0.78
	Average change in scatter for each method of wrapping					
	Single wrap, waxed paper	Double wrap, waxed paper	Single wrap, aluminium foil	Necessary difference		
Blue	−0.013	−0.052	−0.112	0.21		
Green	+0.192	+0.028	−0.177	0.26		
Red	−1.01	−0.768	−1.28	0.43		
Total (Brightness)	−0.832	−0.792	−1.57	0.61		
	Average change in scatter for each method of thawing					
	In brine	In pickle	In air	In water	Necessary difference	
Blue	+0.244	+0.093	−0.217	−0.355	0.24	
Green	+0.638	+0.011	−0.180	−0.411	0.30	
Red	−0.702	−0.151	−1.75	−1.47	0.50	
Total (Brightness)	+0.135	−0.038	−1.61	−1.68	0.70	
	Average change in scatter at each stage of conversion					
	After defrosting	After curing	After maturation	Necessary difference		
Blue	−0.368	−0.118	+0.310	0.21		
Green	−0.270	−0.220	+0.530	0.26		
Red	−2.14	−0.863	−0.050	0.43		
Total (Brightness)	−2.78	−1.20	+0.790	0.61		

curing they were more stable but still darkened significantly, whereas at the end of maturation they became significantly brighter.

The effects of thawing method, stage of conversion, and the interaction of these variables on colour stability is best shown from the detailed results appearing in Table VII. Most of the differences in brightness stability between the various individual treatments are too small to attain significance. Since the colour stability of samples thawed in air and water improved during cure and maturation, the method of thawing has little effect on the stability of the final product. Correlation coefficients computed between the colour stability at different stages were, with one exception, uniformly insignificant. For the air-thawed samples the correlation between colour stability after thawing and after cure exceeded the 5% level of significance ($r = 0.56$).

TABLE VI

ANALYSIS OF VARIANCE OF COLOUR STABILITY MEASUREMENTS

Variance due to:	Mean square				
	D.f.	Blue	Green	Red	Total (Brightness)
Temperature	4	0.64	1.51*	0.78	7.01*
Wrapping	2	0.15	2.04*	3.91	11.4 *
Thawing	3	3.43***	6.58***	23.8 ***	40.6 ***
Stage of development	2	7.06***	12.2 ***	66.6 ***	192 ***
Temperature × wrapping	8	0.45	0.97	2.43	8.25**
Temperature × thawing	12	0.53	1.97***	1.47	16.6 ***
Temperature × stage	8	1.10**	1.65**	0.44	7.15*
Wrapping × thawing	6	0.44	2.46***	0.90	22.0 ***
Wrapping × stage	4	0.46	0.56	0.35	2.52
Thawing × stage	6	3.46***	10.0 ***	27.3 ***	96.6 ***
Error	124	0.33	0.52	1.41	2.82

* *Indicates 5% level of significance compared with residual.*
** *Indicates 1% level of significance compared with residual.*
*** *Indicates 0.1% level of significance compared with residual.*

TABLE VII

EFFECT OF METHOD OF THAWING ON COLOUR STABILITY (BRIGHTNESS) OF LEAN AT VARIOUS STAGES DURING CURE

Thawed in	Change in total scatter, %		
	After thawing	After cure	After maturation
Brine	+0.85	−1.4	+1.1
Pickle	+0.29	−1.1	+0.66
Air	−6.8	−0.57	+0.91
Water	−5.5	−1.7	+0.50

Comparison of Colour of Internal and Exposed Surfaces

In commercial practice the colour of the exposed surface is frequently used to assess the suitability of a given storage or thawing practice. By making certain observations on the colour of these exposed surfaces it was possible to obtain a comparison of the colour on the internal and exposed surfaces of corresponding samples.

The 30 pieces subjected to the various storage treatments had 60 ends exposed during storage. These were divided at random into three groups of 20 for observation after thawing, cure, and maturation, respectively. This yielded surfaces that had been exposed to two or more of the successive processes studied. It was also possible to obtain surfaces, from the corresponding pieces, that had been exposed only to the process immediately preceding the stage at which the measurement was made, i.e., thawing, curing, and maturation or combinations of these factors.

TABLE VIII

DIFFERENCE BETWEEN COLOUR SCATTERS OF EXPOSED AND INTERNAL SURFACES AT VARIOUS STAGES

Stage compared	Surfaces compared†	Difference between mean scatters				Remarks
		Blue	Green	Red	Total	
After thawing	I—Ss.t.	1.62 *	0.835	2.01	4.46	Both surfaces showed similar variability.
After cure	I—Sc.	−1.23 *	−1.76	−1.15	−4.13	Both surfaces showed similar variability.
	Sc—Ss.t.c.	0.875	−0.800	0.210	0.285	
	I—Ss.t.c.	−0.350	−2.56 **	−0.935	−3.84	Surface colour more variable than internal colour in red and total only.
After maturation	I—Sm.	0.130	0.030	−0.380	−0.220	Sm. more variable than I in blue only.
	Sm—Ss.t.c.m.	0.095	−0.655	0.780	0.225	Ss.t.c.m. more variable than Sm. in green only.
	I—Ss.t.c.m.	0.225	−0.625	0.405	0.005	Ss.t.c.m. more variable than I in all colour components.

† I = *Percentage scatter from internal surface;* S = *Percentage scatter from exposed surface, for all treatments indicated by the subscripts, where* s = *storage,* t = *thawing,* c = *cure, and* m = *maturation.*

* *Indicates 5% level of significance.*

** *Indicates 1% level of significance.*

These results are presented in Table VIII as the differences between the scatter of the internal surfaces minus that of the exposed surfaces. For comparisons not involving the internal colour, the scatter for the surface suffering the greatest exposure was subtracted from that of the surface having the least exposure. All differences are algebraic, negative values indicating that the exposed surface was brighter. Statistical methods were used to determine whether the observed differences exceeded the necessary difference computed for the variations within each group of 20 samples, taken at each stage.

It is evident from the table that comparatively few of the differences between the exposed and internal surfaces were statistically significant. Since each group of comparable samples was taken at random from material that had been treated by procedures that had affected the colour significantly, it is obvious that the difference between the internal and external surfaces would have to be large to attain significance. Nevertheless material showing similar variability may be used commercially, and it is of interest to determine whether exposure causes significant changes on such material.

Of the significant differences, exposure during storage and thawing reduced the blue scatter, whereas exposure during cure alone increased the scatter of this component. Surfaces exposed to storage, thawing, and cure showed an increased green scatter over that of the internal surface. The variability,

within groups of internal and exposed surfaces, was comparable until after curing, but subsequently the exposed samples were consistently more variable in one or more of the colour components.

Comparison of Colour and Colour Stability of Smoked and Pale-Dried Bacon

The results of the colour and colour stability measurements on the smoked and pale-dried residues from the previous treatments appear in Table IX. In all instances the pale-dried material was brighter than the smoked material. The two groups of samples did not differ, however, in their average colour stability. Smoking deposits material on the surface of the bacon, but it is difficult to see why this should affect the internal colour of the lean. It is possible that the colour differences were due to temperature effects for although the air temperature and time of exposure were similar for both groups the temperature attained by the samples may have differed owing to the obvious difference in the size of the smoking room and a laboratory oven. Further investigations of this point are under way.

TABLE IX

COMPARISON OF COLOUR AND COLOUR STABILITY OF SMOKED AND PALE-DRIED BACON

Colour measurement	Treatment	Internal colour	Internal colour after exposure 72 hr. at 50° F.	Colour stability
Blue	Smoked	14.58	13.92	−0.65
	Pale-dried	16.50	15.63	−0.87
	Difference	1.92**	1.71**	0.22
Green	Smoked	17.99	17.41	−0.59
	Pale-dried	20.14	19.49	−0.65
	Difference	2.15**	2.08*	0.06
Red	Smoked	28.02	27.21	−0.81
	Pale-dried	31.03	30.03	−0.99
	Difference	3.01**	2.82**	0.18
Total	Smoked	60.59	58.54	−2.05
	Pale-dried	67.68	65.16	−2.52
	Difference	7.09	6.62	0.47

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

Conclusions

The combination of wrapping in an impermeable sealed wrapper and low storage temperature (−29° C.) appears from these results to be ideal for the preservation of the internal colour of pork for storage periods of about one year. Nevertheless the colour changes under other conditions are frequently only small, and storage temperatures of −18° C. with a wrapping, reasonably

moisture resistant, should yield satisfactory results under commercial conditions.

The colour of the pork is significantly altered by the method of thawing. Thawing in brine or pickle produces darker samples than in air or water. Most of the differences observed after thawing, decrease markedly during cure and maturation, so that the final cured product is not seriously affected by the procedure used. Nevertheless thawing in pickle or brine appears to cause colour changes of the same nature that take place during cure. The greater colour permanence at all stages following thawing in pickle suggests that this method is preferable.

The effect of the various treatments on colour stability depends primarily on the effect of these treatments on the initial colour. Under storage conditions that preserve the original colour, and subsequent treatments that yield bright or light coloured samples the product generally shows the greatest instability on exposure. Concerning colour stability in the final matured bacon there is little to choose between pork thawed by the several procedures tested.

There was seldom any significant difference between the colour of the interior of the samples and that of the outside surfaces subjected to one or more of the treatments under investigation. This is attributable in part to the variation in the colour of the pieces following the various treatments. The exposed surfaces, however, were more variable than the interior after curing. Pale-dried bacon was lighter in colour than smoked bacon, but there was no significant difference in their colour stabilities.

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CANADIAN WILTSHIRE BACON

XVII. RANCIDITY IN PORK FAT AFTER FROZEN STORAGE AND CONVERSION TO BACON[1]

By W. Harold White[2]

Abstract

Determination of the peroxide oxygen and free fatty acid content of the fat of pork, stored under various conditions and subsequently converted to bacon, showed that temperature, method of wrapping, and stage in the conversion to bacon were the most important factors governing the oxidation and hydrolysis of the fat. Of the conditions studied, storage at temperatures of −18° to −23° C. with an aluminium foil wrapping, followed by thawing in brine or pickle were the most effective in retarding rancidity. The greatest increase in the peroxide oxygen content of the fat occurred during cure, whereas that of free fatty acid increased at a relatively uniform rate throughout the various conversion steps. Smoking had greater antioxidant effect on the fat than pale-drying. Since in all instances the content of free fatty acid was low, spoilage in pork or bacon fat is primarily due to oxidation.

Introduction

It is frequently necessary to store pork for the subsequent manufacture of Wiltshire bacon. The importance of this problem in Canadian agricultural economy has been outlined in previous publications (1, 3). In one of these (1) the effects of wrapping procedure, storage temperature, and method of thawing on colour and colour stability of the lean meat at various stages in the conversion of pork to bacon were given. The purpose of the present paper is to describe the effects of these same treatments on peroxide oxygen and free fatty acid formation in the fat.

Materials and Methods

The material and treatments employed have been described in detail previously (1). In brief, samples of pork back, wrapped in either a single or double layer of waxed paper or a single layer of aluminium foil and overwrapped with a heavy Kraft paper, were stored in duplicate at temperatures of approximately −7°, −12°, −18°, −23°, and −29° C. (20°, 10°, 0°, −10°, and −20° F.) for 48 weeks. After cutting each back into two pieces, groups of 15 samples each were thawed in air, water, brine, or curing pickle. The samples were then cured in a commercial Wiltshire pickle at 4° C. (39° F.) for three and one-half days, matured for seven days at 3° F. (38° F.) and pale-dried or smoked for 7 hr. at 54° C. (130° F.). A small portion of the back fat was removed from each sample after each of the steps described above. In

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[2] *Biochemist, Food Storage and Transport Investigations.*

addition, since each back was divided into two at the termination of the storage period it was possible to compare the effect of the subsequent treatments on fresh surfaces and on those exposed during storage.

The peroxide oxygen and free fatty acid content of each of the samples of fat were determined by methods briefly described previously (3), and to be given in detail in a future publication. The selection of the two methods employed was based on the hypothesis that peroxide oxygen formation was indicative of oxidative changes whereas the presence of free fatty acid was primarily attributable to hydrolytic alterations in the fat.

Effect of Storage Conditions and Curing on Rancidity

The mean values of the peroxide oxygen and free fatty acid contents of the fat for each temperature, method of wrapping and of thawing, and stage of manufacture, averaged over all samples for the condition indicated, are given in Table I. The progressive increases in peroxide oxygen content noted for temperature changes from $-18°$ to $-12°$, and from $-12°$ to $-7°$ C. all reached the level of statistical significance. The fat of pork stored at $-7°$ C. and subsequently converted to bacon was definitely rancid; that held at $-12°$ C. was well advanced in its induction period of rancidity. The values obtained for the lower temperatures were small, and approximate those of cured fresh pork. For periods comparable to that employed here a storage temperature of $-18°$ C. is essential if spoilage of pork fat is to be avoided. Little apparent advantage is to be gained by the use of lower temperatures.

The free fatty acid content likewise significantly increased with increase in temperature. However, the concentrations present even at the higher temperatures were relatively low, and suggest that spoilage due to the formation of fatty acids is normally of little importance for pork fat.

The differences in peroxide oxygen content observed for each of the three types of wrappings were significant. Although a double wrap of waxed glassine paper was slightly more effective than a single wrapping in retarding oxidative rancidity, the impermeable aluminium foil covering was much superior to both these types. In contrast to peroxide oxygen, the free fatty acid content was greatest for those samples wrapped in aluminium foil, and least for those in the single wrap of waxed paper.

The differences in peroxide oxygen content observed between samples subjected to the various methods of thawing were not significant when considered for the experiment as a whole. However, thawing in brine or pickle usually yielded a product with slightly less peroxide oxygen than thawing in air or water. The explanation of this behaviour of the peroxide oxygen formation is not apparent. It may be that the lower solubility of oxygen in brine or pickle, as compared to water, or air itself, exercised a retarding effect. With regard to the content of free fatty acid, the results were similar. In this instance thawing in pickle gave a significantly lower mean value than in brine, which in turn was significantly lower than in air or water.

TABLE I

PEROXIDE OXYGEN AND FREE FATTY ACID CONTENT OF THE FAT OF PORK STORED AND CONVERTED TO BACON UNDER VARIOUS CONDITIONS

Factor	Peroxide oxygen[1]	Free fatty acid[2]
	Mean	Mean
Temperature, °C.		
−7	31.8	2.09
−12	5.84	0.93
−18	2.27	0.64
−23	1.22	0.50
−29	1.33	0.62
Necessary difference	1.77	0.05
Method of wrapping		
Single wrap, waxed paper	11.1	0.78
Double wrap, waxed paper	9.47	0.82
Single wrap, aluminium foil	4.95	1.27
Necessary difference	1.37	0.04
Stage of manufacture		
After thawing	7.21	0.88
After cure	9.13	0.92
After maturation	9.16	1.06
Necessary difference	1.37	0.04
Method of thawing		
In air	8.73	1.05
In water	9.32	1.01
In brine	7.40	0.91
In pickle	8.54	0.85
Necessary difference	—	0.05

[1] *Mean content as ml. 0.002N sodium thiosulphate over all conditions studied other than that indicated.*

[2] *Mean content as per cent oleic acid over all conditions studied other than that indicated.*

Although the mean values of peroxide oxygen after maturation and after cure did not differ significantly, both were significantly greater that that obtained after thawing. Oxidative changes in the fat were therefore most extensive during cure. This may be due to the pro-oxidant effect of nitrite present in the curing pickle, or to the activation of the enzyme lipoxidase (4) by sodium chloride. Although significant increases in free fatty acid occurred between all three stages studied, the greatest change was during the maturation period.

The relative importance of the factors studied, in effecting changes in the peroxide oxygen and free fatty acid content, was assessed by means of an analysis of variance. Differences in the peroxide oxygen content, attributable to variations in temperature, wrapping, and stage of conversion, all

reached the level of statistical significance (Table II), whereas the method of thawing had little effect. Of the factors studied here, storage temperature and method of wrapping were of greatest importance. Similar general conclusions were reached with respect to the free fatty acid content (Table III). However, in this instance the method of thawing also had a significant effect.

Of the differential quantities, those relating temperature with the other three factors were usually of greatest influence. These have been graphically illustrated for both peroxide oxygen and free fatty acid in Figure I. The

TABLE II

ANALYSIS OF VARIANCE FOR THE PEROXIDE OXYGEN CONTENT OF THE FAT OF PORK STORED AND CONVERTED TO BACON UNDER VARIOUS CONDITIONS

Variance attributable to:	D.f.	Mean square
Temperature	4	6251 ***
Wrapping	2	606 ***
Thawing	3	28.8
Stage of conversion	2	75.2**
Temperature × wrapping	8	448 ***
Temperature × thawing	12	26.8*
Temperature × stage	8	35.7*
Wrapping × thawing	6	43.9**
Wrapping × stage	4	28.3
Thawing × stage	6	42.3*
Error	124	14.4

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*
*** *Indicates 0.1% level of significance.*

TABLE III

ANALYSIS OF VARIANCE FOR THE FREE FATTY ACID CONTENT OF THE FAT OF PORK STORED AND CONVERTED TO BACON UNDER VARIOUS CONDITIONS

Variance attributable to:	D.f.	Mean square
Temperature	4	15.4 ***
Wrapping	2	4.42 ***
Thawing	3	0.396***
Stage of conversion	2	0.543***
Temperature × wrapping	8	0.253***
Temperature × thawing	12	0.221***
Temperature × stage	8	0.031*
Wrapping × thawing	6	0.032*
Wrapping × stage	4	0.015
Thawing × stage	6	0.005
Error	124	0.013

* *Indicates 5% level of significance.*
*** *Indicates 0.1% level of significance.*

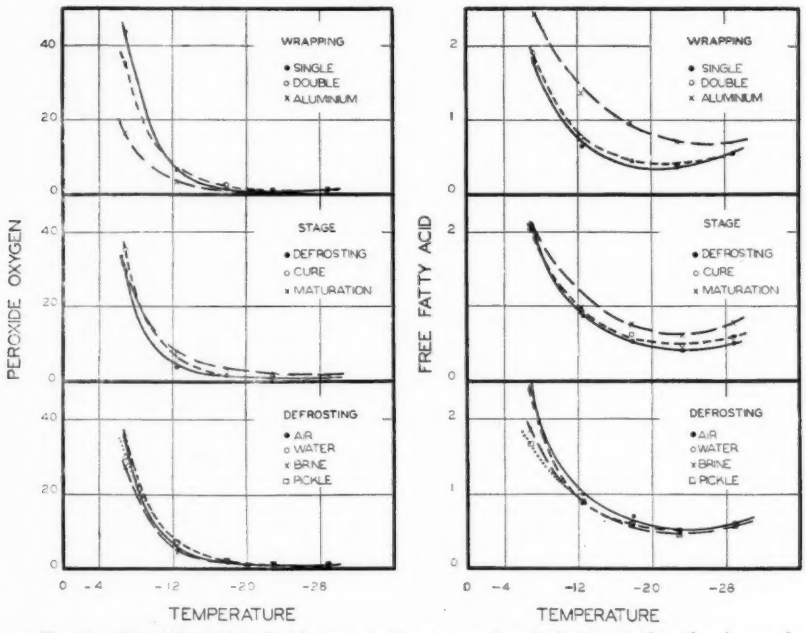

FIG. 1. *The differential effect between temperature and method of wrapping, thawing, and stage of conversion of pork to bacon on peroxide oxygen and free fatty acid formation in pork and bacon fat.*

curves show that the variations in the method of wrapping, thawing, etc. exerted their maximum effect on material stored at the higher temperatures, and that their influence decreased progressively with decrease in temperature. Since the fat of samples stored at $-7°$ C. was well advanced in its induction period of rancidity and therefore especially susceptible to subsequent differences in treatment, these samples contributed much of the variance responsible for the significant interrelations.

The effect of position of sampling (internal or external, as previously described) was determined by the computation of t tests of significance between the two positions for each sample at all three stages. As would be expected the external samples in each instance contained significantly more peroxide oxygen than those from the internal position (Table IV). In addition the magnitude of the difference increased progressively with stage.

Position of sampling exerted an opposite effect on the free fatty acid content. For each stage the samples from the internal position contained significantly greater concentrations of free fatty acid than those obtained externally. This would suggest that oxidative changes contribute little to free fatty acid formation. However, the actual magnitude of the differences observed in free fatty acid content were small, and of little practical significance.

TABLE IV

t TESTS OF SIGNIFICANCE ON EFFECT OF POSITION FOR THE PEROXIDE OXYGEN AND FREE FATTY ACID CONTENT OF THE FAT OF PORK AT VARIOUS STAGES IN CONVERSION TO BACON

Property	Stage of conversion	Position with greater concentration	Mean difference	*t*
Peroxide oxygen[1]	After defrosting	External	4.76	2.65*
	After cure	External	4.72	2.87**
	After maturation	External	6.55	4.15**
Free fatty acid[2]	After drefosting	Internal	0.12	2.35*
	After cure	Internal	0.26	5.95**
	After maturation	Internal	0.06	1.07

[1] *As ml. 0.002N sodium thiosulphate.*
[2] *As per cent oleic acid.*
* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

Comparative Effect of Smoking and Pale-drying on Rancidity

When the free fatty acid and peroxide oxygen contents of the fat after smoking or pale-drying were compared by means of *t* tests with the corresponding values for after maturation, it was observed that in both instances significant increases had occurred (Table V). A comparison of the values for pale-dried samples alone with those for the corresponding samples after maturation showed that the peroxide oxygen and free fatty acid content had both increased significantly during pale-drying. A similar comparison for smoked samples showed that, although both the peroxide oxygen and free fatty acid content

TABLE V

t TESTS OF SIGNIFICANCE ON THE EFFECT OF SMOKING AND PALE-DRYING OF BACON ON THE PEROXIDE OXYGEN AND FREE FATTY ACID CONTENT OF THE FAT

Treatments compared	Treatment giving greater content	Mean difference	*t*
Peroxide oxygen content[1] at:			
After smoking or pale-drying with after maturation	After pale-drying or smoking	3.15	4.39**
After pale-drying with after maturation	After pale-drying	4.82	4.07**
After smoking with after maturation	After smoking	1.48	1.90
Free fatty acid content[2] at:			
After smoking or pale-drying with after maturation	After pale-drying or smoking	0.28	9.43**
After pale-drying with after maturation	After pale-drying	0.23	6.55**
After smoking with after maturation	After smoking	0.34	7.15**

[1] *As ml. 0.002N sodium thiosulphate.*
[2] *As per cent oleic acid.*
** *Indicates 1% level of significance.*

had increased, only that for free fatty acid reached the level of statistical significance. The peroxide oxygen content of the pale-dried samples was slightly greater than that of the smoked, but not significantly so. Smoked and pale-dried samples possessed approximately the same concentrations of free fatty acid.

Discussion and Conclusion

Although the reason for the variations in peroxide oxygen content as affected by the wrapping methods studied is unknown, it may be due either to the direct effect of greater exclusion of oxygen by the aluminium foil, or, indirectly, to the higher relative humidity within this form of wrapping. The presence of a high relative humidity may be associated with a retardation of surface desiccation, which causes a reduction in the effective area exposed to the air, and consequently of the rate of diffusion of oxygen into the fat. If the enhanced protection obtained by the aluminium foil were due merely to the exclusion of oxygen it might reasonably be expected to be independent of temperature. However, temperature had a significant effect on oxidation in the fat. Moreover, the quantity of oxygen required to cause rancidity in pork fat is small, and it may be reasonably assumed that more than sufficient would be present within any of the wrappings employed here. Consequently, it is considered that the development of rancidity in pork fat, as indicated by peroxide oxygen formation, at temperatures used in frozen storage, is retarded by high relative humidities. A similar observation was noted previously for frozen poultry (2).

The observed effect of wrapping on free fatty acid formation may be due to the progressive increase in relative humidity from the single waxed paper to the aluminium foil wrap, and its consequent effect on the growth of micro-organisms capable of effecting lipolysis of the fat. If so, the effect of wrapping should be greatest at the higher storage temperatures, which are most favourable for the growth of micro-organisms. Data given in Table VI for the mean values of free fatty acid for each wrapping at each of the temperatures studied shows this to be essentially true.

TABLE VI

MEAN VALUES OF THE FREE FATTY ACID CONTENT OF PORK FAT AFTER THAWING, FOR VARIOUS STORAGE CONDITIONS STUDIED

Temperature, °C.	Wrapping procedure		
	Single waxed paper	Double waxed paper	Aluminium foil
− 7	1.79†	1.94	2.53
−12	0.61	0.72	1.33
−18	0.42	0.33	0.86
−23	0.30	0.31	0.58
−27	0.46	0.45	0.58

† *All values of free fatty acid as per cent oleic acid.*

Since the peroxide oxygen content increased significantly after pale-drying but not after smoking, the smoke must have contained antioxidants. This is of interest since the possibility existed that the antioxidant effect of smoking might be due primarily to the inactivation of the oxidizing enzyme, lipoxidase, by heat. Although this investigation does not specifically show the effect of heat, since no tests were made on unheated bacon, the results indicate the antioxidant action of the smoking process is due at least in part to factors other than temperature.

From the results presented here it may be concluded that pork should be stored at temperatures of $-18°$ to $-23°$ C. for a period comparable to that employed here. Considerable protection from atmospheric oxidation is to be obtained by the use of moisture-impervious wrapping materials, such as aluminium foil. Although a double wrap of waxed paper was slightly better than a single wrap, it is doubtful whether it gave sufficient added protection to merit the extra cost. If frozen stored pork is to be subsequently converted to bacon, it should be thawed in a curing pickle or brine solution rather than in air or water. Since the over all changes in the free fatty acid content were low, deterioration in pork or bacon fat as the result of hydrolytic changes would appear to be of little importance. Decomposition due to oxidation is therefore primarily responsible for spoilage.

Acknowledgments

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CANADIAN WILTSHIRE BACON

XVIII. EFFECT OF TEMPERATURE AND BACTERIAL GROWTH ON NITRITE CONTENT[1]

By W. Harold White[2] and N. E. Gibbons[3]

Abstract

Samples of bacon, with bacterial populations adjusted to two levels, were held at temperatures of 4°, 21°, 38°, and 55° C. for 20, 40, 80, and 160 hr. Both the total number of organisms and the number capable of reducing nitrate to nitrite were significantly correlated with the nitrite content. Furthermore, the samples adjusted to the high bacterial level usually contained more nitrite after treatment than those from the same hog but containing fewer bacteria. The increase in nitrite, observed here and previously, at temperatures below 55° C., is attributable primarily to bacterial growth and not to enzymes or other constituents of the bacon.

Introduction

In a previous investigation (6), it was found that, as in the smoking or pale-drying processes, the nitrite content of small cuts of bacon increased upon heating at temperatures below 55° C. Since the amount of nitrite affects both the colour and colour stability of bacon (7, 8), further studies were undertaken to determine the factors responsible for the increase in nitrite content.

It was suggested in a previous paper (6) that the growth of bacteria might be responsible for the increases in nitrite. In support of this it was observed that, upon heating, the nitrite content reached a maximum at 40° C., a temperature at which rapid bacterial growth might be expected. Furthermore, comparable increases did not occur in whole sides (1), possibly because of the greater opportunity for contamination and the greater exposure of the substrate to bacterial action in the small pieces. However, it was possible that certain enzymes or other constituents or properties of the meat were responsible for the reduction of nitrate. The present investigation was undertaken to determine the extent to which bacteria were responsible for the observed changes, and at the same time to obtain indirect evidence of the importance of other agencies.

Material and Methods

The material consisted of two rib-in export backs removed from each of three hogs that had been cured together in the same tank and treated as nearly alike as possible within the limitations of commercial plant practice.

After cure the six backs were incubated for two days at 20° C. in order to increase the bacterial population, while maintaining comparable nitrite levels.

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In order to ensure the existence of two bacterial levels, the ribs of three sides, selected so that each hog was represented, were scraped, and the exposed lean surface scrubbed and washed with sterile 4% sodium chloride solution. The lean portion of each of the three backs was ground and mixed by several passages through a sterile food chopper in a room at 4° C. The lean from each of the remaining backs, without washing or scrubbing, was mixed with the surface scrapings and ground to give three samples of higher bacterial content.

Fifty-gram subsamples of the ground material from each of the six backs were placed in sterile, sealed, glass containers, and held in air ovens at temperatures of 4°, 21°, 38°, and 55° C. for 20, 40, 80, and 160 hr. In addition to these 96 samples, subsamples of each ground back were taken at the beginning, middle, and end of the weighing procedure in order to obtain representative values for the initial bacterial and nitrite content. The selection of temperatures was based on the following considerations: 4° C. represented the normal temperature for curing and maturation; 21° C. was the temperature previously used for bacteriological studies on bacon (2); at 38° C., maximum nitrite production was noted previously; a temperature of 55° C. had been previously found to have little effect on the nitrite content (6). At the termination of the desired treatment the samples were brought rapidly to room temperature (approximately 20° C.), weighed, thoroughly mixed, and sampled for bacteriological study. They were then frozen immediately and stored at −40° C. until analysed chemically.

The nitrite content of the meat after the various treatments was determined in duplicate by methods previously described (5). For bacteriological examination duplicate 5-gm. samples of the ground meat (10 gm. for the initial samples) were shaken 200 times in bottles containing 4% sodium chloride solution and coarse silica sand. All subsequent dilutions were made with 4% sodium chloride solution. Agar containing 4% sodium chloride was used throughout (2). Plates were incubated at 20° C. for six days. However, at the final sampling duplicate sets of plates for the meat samples held at 38° C. and 55° C. were also incubated at the corresponding temperatures for three days.

To determine the proportion of the total bacterial population capable of reducing nitrate, all of the colonies from a plate or section of a plate were inoculated directly into beef extract broth containing 4% sodium chloride and 0.1% potassium nitrate. After four-days' incubation at 20° C. these tubes were tested for the presence of nitrite in the customary manner (3). If no nitrite was present, a test was made for nitrate by reduction with zinc dust (3) to determine whether reduction had not occurred, or whether it had been carried beyond the nitrite stage. Of some 3700 cultures examined only four reduced all of the nitrate beyond the nitrite stage. Although 100 colonies were usually selected for each test, this was at times impossible. However, unless 25 colonies were available the results are not presented. The proportion of nitrate-reducing organisms was estimated from tubes showing definite growth.

Results

The results for the effect of temperature on the nitrite content of Wiltshire bacon, presented as the means of the six sides regardless of bacterial level (Table I), show close agreement with those obtained in the previous investigation (6). However, the actual magnitude of the present changes was somewhat greater than of those noted previously. This is presumably due to a greater average bacterial population and to the relatively greater area of exposed surface.

TABLE I

MEAN NITRITE CONTENT OF BACON AFTER VARIOUS HEAT TREATMENTS (AS P.P.M. ON DRY MATTER BASIS)

Period of heat treatment, hr.	Temperature of heat treatment, °C.			
	4	21	38	55
0	51	51	51	51
20	48	359	3249	23
40	51	1403	2966	27
80	49	2481	3079	149
160	110	4082	1549	304

Except in the 160-hr. treatment, the nitrite content increased with increase of temperature to a maximum at 38° C., and then decreased to approximately the original level, at 55° C. However, as the length of the heating period increased, the differences in content between 21° and 38° C. decreased, until at 160 hr. the greatest value was obtained at 21° C.

The total number of organisms showed the same behaviour as the nitrite content (Table II). The most rapid bacterial development occurred at 38° C., reaching a maximum between 80 and 160 hr. At 4° and 21° C., the number of bacteria continued to increase, attaining the greatest observed value after 160 hr.

TABLE II

MEAN TOTAL BACTERIAL CONTENT OF BACON AFTER VARIOUS HEAT TREATMENTS (AS LOGARITHM OF NUMBER OF ORGANISMS PER GM. OF MEAT)

Period of heat treatment, hr.	Temperature of heat treatment, °C.			
	4	21	38	55
0	5.74	5.74	5.74	5.74
20	5.90	7.46	7.70	1.30
40	6.02	7.72	7.76	5.19
80	6.16	7.93	9.08	4.38
160	8.07	8.93	8.60	3.42
160	—	—	8.75[1]	4.37[2]

[1] *Plates incubated at 38° C.*
[2] *Plates incubated at 55° C.*

TABLE III

NITRITE CONTENT, AS P.P.M., AND BACTERIAL CONTENT, AS LOGARITHM OF NUMBER OF ORGANISMS PER GM., OF BACON HEATED AT 55° C. FOR VARIOUS PERIODS

Time of treatment, hr.	Sample											
	1		2		3		4		5		6	
	Nitrite	Bacteria	Nitrite	Bacteria	Nitrite	Bacteria	Nitrite	Bacteria	Nitrite	Bacteria	Nitrite	Bacteria
20	27	<1.3	30	<1.3	25	<1.3	19	<1.3	24	<1.3	15	<1.3
40	20	6.41	24	6.91	12	<1.3	38	7.69	57	7.52	9	<1.3
80	778	8.35	34	>7.0	65	>7.0	6	<1.3	3	<1.3	6	<1.3
160	6	<3.3	6	<3.3	5	<3.3	393	<1.3[1]	9	<1.3	1406	8.00

[1] *Count at 55° C. = 6.23.*

There was considerable variation in the bacterial and nitrite content of the samples held at 55° C. In general the number of organisms decreased rapidly. However, at 40 hr., four samples, 80 hr., three samples, and 160 hr., one sample had high counts (Table III). One sample, held for 160 hr., had a low count, as shown on plates incubated at 20° C., but a high count on plates incubated at 55° C. This was the only instance in which plates incubated at either 38° or 55° C. had an appreciably different count from those incubated at 20° C. The plates with high counts were practically pure cultures of a spore-bearing, nitrate-reducing rod. This organism, which was apparently scattered sparingly through the original samples and of a type rarely found in bacon, accounted for the high nitrite values and high bacterial counts observed after 80 and 160 hr. Actually the only samples with high nitrite contents were those having high bacterial counts.

The percentages of the total number of organisms capable of reducing nitrate to nitrite are shown in Table IV. Not only were the majority of the organisms able to reduce nitrate to nitrite, but the number of reducing organ-

TABLE IV

PERCENTAGE OF TOTAL NUMBER OF ORGANISMS CAPABLE OF REDUCING NITRATE TO NITRITE IN BACON AFTER VARIOUS HEAT TREATMENTS

Period of heat treatment, hr.	Temperature of treatment, °C.			
	4	21	38	55
0	91	91	91	91
20	86	91	74	—
40	90	96	98	100
80	84	79	47	—
160	90	67	85[1]	—

[1] *83% when incubated at 38° C.*

isms was highly correlated with the total number ($r = 0.95$, which exceeds the 1% level of significance for the 66 degrees of freedom available). Although the total count was obtained by accepted aerobic procedures, conditions existing in the determination of nitrate reduction and in the meat ranged from aerobic to essentially anaerobic. It has been stated (4) that nitrate is reduced by pure cultures of organisms under aerobic or anaerobic conditions and it would appear that reduction takes place under both conditions in meat.

In Fig. 1 the mean values of the nitrite content and total count for each of the two bacterial levels are plotted against temperature for each of the periods studied. The close similarity in the nature of the curves for the nitrite and bacterial content is evident. Moreover, the samples adjusted to the high bacterial level contained more nitrite in most instances than similar samples containing fewer bacteria, although the difference between the two levels in the original samples was not large (factor of 4 to 6).

The magnitude of the apparent inter-relation between the nitrite content and the bacterial count was determined by means of simple coefficients of correlation. Such a computation for nitrite and total bacterial content indicated that the two quantities were significantly correlated (Table V).

TABLE V

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN THE NITRITE CONTENT AND TOTAL NUMBER OF ORGANISMS IN BACON AFTER VARIOUS HEAT TREATMENTS

QUANTITIES CORRELATED	D.f.	r
Logarithm of nitrite content with logarithm of total number of organisms for the following conditions:		
All treatments	94	0.72**
All treatments less 160 hr.	70	0.76**
All treatments less 80 and 160 hr.	46	0.69**
All treatments less 55° C.	70	0.69**
All treatments less 4° and 55° C.	46	0.44**
All treatments less 38° and 55° C.	46	0.68**
All treatments less 160 hr. at 55° C.	52	0.84**
All treatments less 160 hr. at 4° and 55° C.	34	0.47**
All treatments less 160 hr. at 38° and 55° C.	34	0.80**
All treatments less 80 and 160 hr. at 55° C.	34	0.85**
All treatments less 80 and 160 hr. at 4° and 55° C.	22	0.83**
All treatments less 80 and 160 hr. at 38° and 55° C.	22	0.80**
All treatments less { 4° and 55° at 20, 40, and 80 hr. 4°, 38°, and 55° C. at 160 hr.	40	0.71**
All treatments less { 4° and 55° at 20 and 40 hr. 4°, 38°, and 55° at 80 and 160 hr.	34	0.52**
Logarithm of increase in nitrite content with logarithm of increase in total number of organisms for the following conditions:		
All treatments less { 20, 40, and 80 hr. at 55° C. 160 hr. at 38° and 55° C.	43	0.61**
All treatments less { 4° and 55° C. at 20, 40, and 80 hr. 4°, 38°, and 55° C. at 160 hr.	40	0.54**
All treatments less { 4° and 55° C. at 20 and 40 hr. 4°, 38°, and 55° C. at 80 and 160 hr.	34	0.52**

** *Indicates 1% level of significance.*

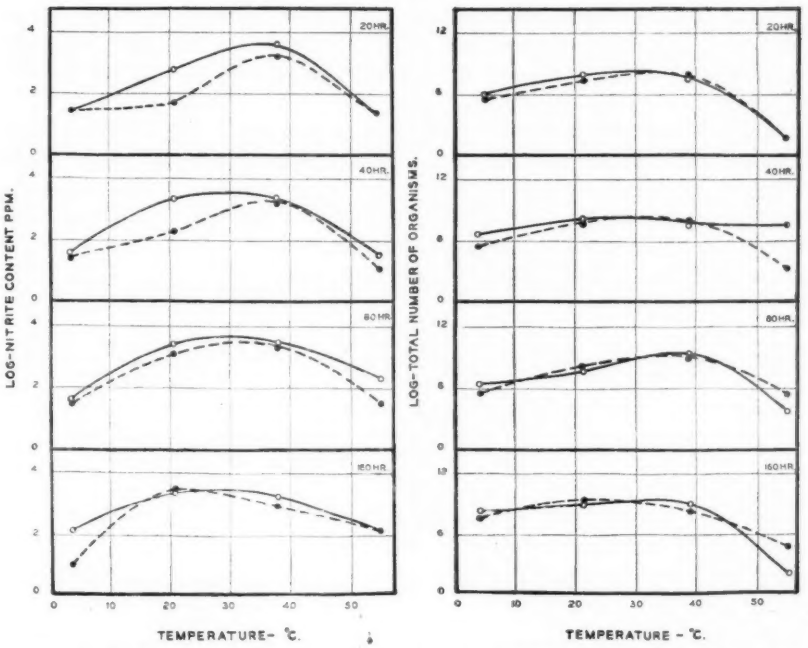

FIG. 1. *Effect of time and temperature on the nitrite content and total bacterial count of Wiltshire bacon.* ——— *Samples adjusted to high bacterial count.* - - - - - *Samples adjusted to low bacterial count.*

However, only about half of the observed variance was accounted for by this correlation. Since the nitrite present may react with the meat proteins and pigments, be oxidized to nitrate, or reduced to other compounds of nitrogen, it is apparent that the value obtained experimentally is dependent on these factors as well as bacterial reduction from nitrate. Moreover, it may reasonably be assumed that both temperature and time have a differential effect on the reactions causing a decrease in the nitrite content.

Evidence of the differential effects due to time and temperature are shown by the results in Table III, and by the fact that the greatest values for nitrite and bacterial content occurred at 38° C. for the shorter periods of treatments but at 21° C. for the 160-hr. period. More definite proof was obtained by computing simple coefficients of correlation, in which the data obtained for one or more of the conditions was omitted (Table V). Although it is unnecessary to discuss these at length, it is of interest to note that the highest correlations were obtained when the data for heating at 55° C. for 80 and 160 hr. were excluded. This suggests that the greatest decomposition of nitrite occurred under these conditions. The magnitude of the correlation coefficient was such that 72% of the variance was accounted for. Since the combined sampling and experimental errors could account for most of the residual

variance, it is concluded that the formation of nitrite and bacterial growth in bacon are closely related. Statistically significant relations were also obtained between the logarithms of the arithmetic increases in nitrite and total bacterial content.

The results of calculations of correlation coefficients between the nitrite content and the number of nitrate-reducing organisms and between the increases in the logarithms of these quantities confirm in general the above described observations (Table VI). The smaller magnitude of these coefficients is presumably due to the greater experimental error introduced in the determination of those organisms capable of reducing nitrate.

TABLE VI

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN THE NITRITE CONTENT AND NUMBER OF NITRATE-REDUCING ORGANISMS IN BACON AFTER VARIOUS HEAT TREATMENTS

Quantities correlated	D.f.	r
Logarithm of nitrite content with logarithm of number of nitrate-reducing organisms for the following conditions:		
All treatments[1] less 160 hr.	47	0.77**
All treatments[1] less 80 and 160 hr.	28	0.68**
All treatments[1] less 55° C.	60	0.67**
All treatments[1] less 4° and 55° C.	37	0.41*
All treatments[1] less 38° and 55° C.	41	0.67**
All treatments[1] less 160 hr. at 55° C.	42	0.83**
All treatments[1] less 160 hr. at 4° and 55° C.	27	0.49**
All treatments[1] less 160 hr. at 38° and 55° C.	29	0.79**
All treatments[1] less 80 and 160 hr. at 55° C.	24	0.81**
All treatments[1] less 80 and 160 hr. at 4° and 55° C.	13	0.68**
All treatments[1] less 80 and 160 hr. at 38° and 55° C.	17	0.79**
All treatments[1] less { 4° and 55° C. at 20, 40 and 80 hr. 4°, 38°, and 55° C. at 160 hr.	31	0.35*
All treatments[1] less { 4° and 55° C. at 20 and 40 hr. 4°, 38°, and 55° C. at 80 and 160 hr.	25	0.48*
Samples in which nitrite content increased	43	0.79**
Increase in logarithm of nitrite content with increase in logarithm of nitrate-reducing organisms for the following conditions:		
All treatments less 55° C.	41	0.40**
All treatments less 160 hr. at 55° C.	26	0.45*
All treatments less 160 hr. at 38° and 55° C.	13	0.45
All treatments less 80 and 160 hr. at 55° C.	12	0.18
All treatments less 80 and 160 hr. at 38° and 55° C.	5	0.38

[1] *Data for periods at 55° C. incomplete.*

* *Indicates 5% level of significance.*

** *Indicates 1% level of significance.*

Although it has been shown conclusively that the contents of nitrite and bacteria were closely correlated, the possibility existed that conditions favouring the growth of bacteria might also be most suitable for nitrite production by some other agency, e.g., a tissue enzyme of bacon. However, since samples treated to give a higher bacterial content usually contained greater quantities

of nitrite than those from the same hog adjusted to a lower bacterial level, and since at 55° C. the amount of nitrite decreased except where growth of the more thermophilic organisms occurred, it is considered that this possibility is excluded. The increase in nitrite at temperatures below 55° C. observed in this and a previous investigation (6) must then be due primarily to reduction from nitrate by the action of bacteria.

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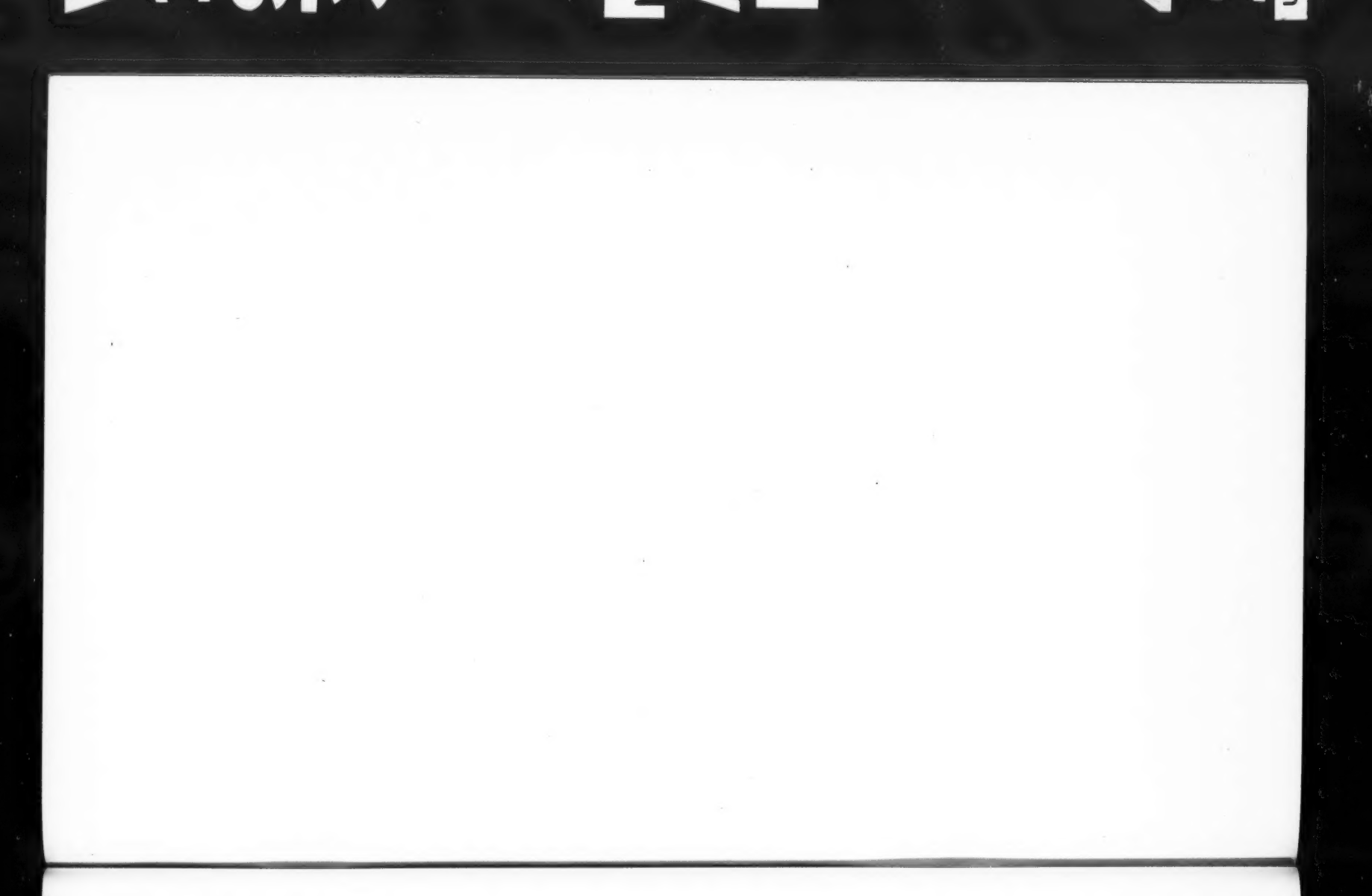

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